AN ABSTRACT OF THE THESIS OF

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Diane J. Carroll

The goal of these experiments was to ensile combinations of shrimp or crab waste with perennial ryegrass straw and analyze it for silage quality and ruminal degradability. The rapid deterioration of seafood wastes required initial preservation prior to ensiling to minimize odor production and protein breakdown. Eight experiments were conducted to preserve, ensile and test the ruminal degradability of shrimp and crab waste. The first three experiments attempted to preserve crab waste with the addition of 0 to 15% molasses for 14 days or shrimp waste with molasses at 0 to 25% for 6 or 21 days. In all three experiments, the addition of molasses decreased crude protein, crude protein loss, and pH, and improved odors. Although a lactic acid fermentation began, the high pH and acetic and butyric acid concentrations indicate fermentation was not completed. The crude protein loss and odor production warrants a quicker method of preservation. In Experiments 4 and 5, shrimp or crab waste were combined with 6 levels of salt from 0 to 12.5% for 6 days. The addition of salt decreased crude protein percentage, crude protein loss and all volatile fatty acid concentrations while increasing the pH and improving the odors. The 7.5% salt-preserved shrimp and crab wastes had 29.9 and 30.7% crude protein, respectively.

Experiments 6 and 7 ensiled the salt-preserved shrimp and crab waste with grass straw, molasses and an inoculant in 15 L mini-silos for 40 days. Shrimp waste was combined at 37, 47 or 52% dry matter while crab waste was combined at 43, 46 or 52% dry matter. Molasses at 0, 10, or 20% and an inoculant were added to both silages. The 47% dry matter, 20% molasses non-inoculated shrimp silage had the following

analysis on a dry matter basis: crude protein, 16.1%, acid detergent fiber, 27.0%, acetic acid, 1.37%, butyric acid, .01%, lactic acid, 7.17%, and a pH of 7.0 and a good silage odor. The 46% dry matter, 10% molasses, inoculated crab silage had the following analysis on a dry matter basis: crude protein, 21.1%, acid detergent fiber, 19.6%, acetic acid, 1.68, butyric acid, .00%, lactic acid, 2.86% and a questionable odor score.

In Experiment 8, the ruminal degradability of shrimp and crab waste and shrimp and crab silage were tested using two fistulated steers and sampled at nine time points ranging from 0 to 96 hr.

Shrimp waste had a ruminally available DM%, CP%, ADF%, and %ADIN of 36.9, 43.0, 44.2, and 43.6% respectively. Crab waste had a ruminally available DM%, CP%, ADF%, and %ADIN of 38.9, 53.3, 41.0, and 33.0% respectively. Ensiling the shrimp and crab wastes improved the percentage of ruminally available DM and CP and lowered the percentage of ruminally available ADF and ADIN. This indicates that ensiling promotes the degradation of chitin and the release of nitrogen to the rumen. Effective preservation of shrimp and crab waste with salt and then adequate fermentation of the wastes with grass straw, molasses and inoculant has increased the ruminal degradability of the wastes and led to successful transformation of the waste into a dairy heifer feedstuff.

TRANSFORMING SHRIMP AND CRAB WASTE INTO DAIRY HEIFER FEED

by

Dale J. Evers

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INTRODUCTION

The growth in world population and other factors have changed America's once plentiful natural resources into scarce, limited commodities. It has become the responsibility of those who manage natural resources to maximize productivity while minimizing the demand on restricted resources. By-products and recycling of nutrients have become commonplace in areas associated with production animal agriculture. A by-product which could be more efficiently utilized in coastal regions is the waste generated from shrimp and crab processing.

Large quantities of shrimp and crab processing waste are produced for which there is no readily available method of disposal. These wastes, which are high in crude protein (CP) and extremely perishable, can be efficiently utilized by ruminant animals. Past work has shown that fresh crab waste can be successfully ensiled with molasses and wheat straw (Abazinge et al., 1994). Shrimp and crab waste produced in western Oregon could conceivably be consumed by the 30,000 dairy heifers raised in Oregon. However, the small amount of waste produced here on a daily basis is insufficient to allow large scale immediate ensiling. The waste would need to be stockpiled for 3-5 days to create such a quantity. Stockpiling would cause protein degradation and odor production, therefore a method of quickly preserving the waste is necessary. Once the waste was preserved it could be ensiled and transformed into a feedstuff palatable to dairy heifers.

The following thesis includes a review of the literature associated with shrimp and crab waste processing, past work on preserving and ensiling seafood waste products, and other factors important to applying of this research. Chapter 2 includes research results from experiments designed to preserve shrimp and crab waste. Chapter 3 discusses results from experiments designed to ensile the preserved shrimp and crab waste followed by a study which tested the degradability of shrimp and crab waste and shrimp and crab silage in the rumen.

1. REVIEW OF LITERATURE

SHRIMP WASTE

Overview of Shrimp Waste Production

A typical harvest season for Pink shrimp (*Pandalus sp.*) on the Oregon coast is from April 15 through October with the catch being evenly distributed throughout the season (Hilderbrand, 1992). In 1992, 9,682 metric tons of shrimp were harvested in Oregon. Approximately 80% of the shrimp landed in Oregon is processed within the state (Costa, 1978). Of the 7,745 metric tons of shrimp processed, about 70% (5,418 metric tons) was disposed of as waste. Approximately 50% (3,873 metric tons) was disposed of on the Oregon coast as solid shrimp waste and 20% (1,545 metric tons) was collected as liquid shrimp waste. The water soluble portion (20%) was diluted approximately 480:1 with processing water and then released into the estuaries (Hilderbrand, 1993). The average annual shrimp harvest in Oregon from 1970 through 1992 was 12,727 metric tons. On average about 50% of the total catch, or 6,364 metric tons is disposed of as waste on the Oregon coast (Hilderbrand, 1993). The annual average U.S. catch from 1984 through 1987 was 157,241 metric tons, with nearly 70% (110,068 metric tons) disposed of as waste (Holliday, 1990).

Chemical Composition

The composition of shrimp waste will vary significantly depending upon the nature of the processing operation. The most common methods of processing are hand-deheading and mechanical-peeling. The waste produced from each process varies in the level of chitin, protein, non-protein nitrogen, and calcium carbonate ($CaCO_3$) present (Meyers and Benjamin, 1987; Bataille and Bataille, 1983; Table 1.1). Mechanical peeling produces a waste with decreased protein and chitin

concentrations and increased $CaCO_3$ levels (Bataille and Bataille, 1983; Table 1.1). Sabry (1992) reported shrimp shell waste to contain 27.9% CP, 21.4% chitin, and 40.0% $CaCO_3$. Table 1.2 lists the complete analysis of shrimp shell waste.

Meyers (1986) separated shrimp waste into it's major components: shrimp heads and shrimp shells. The analysis showed that shrimp heads had a higher percentage of nitrogen corrected protein and percentage of fat, but were considerably lower in percentage of chitin, ash, calcium and phosphorus compared to shrimp shells (Meyers, 1986; Table 1.3).

Table 1.1 Solid shrimp waste composition (Bataille and Bataille, 1983)

	Composition, %		
Source	Protein	Chitin	Calcium carbonate
Hand picking	27.2	57.5	15.3
Mechanical peeling	22.0	42.3	35.7

Disposal

Dumping in landfills has served as a major means of disposal until recent years. Rapid decomposition and odor production has led to the closure of landfills to seafood waste in most areas. Although legally permitted, the rising costs of landfills is becoming prohibitive as an economical means of disposal. Current alternative uses for shrimp waste include the following:

1. soil amendments and compost;
2. shrimp meal;
3. feedstuff for aquatic animals; and
4. chitosan extract.

Table 1.2 Chemical composition of shrimp-shell waste (Sabry, 1992).

Constituents	
Moisture content, %	20.0
	DM basis
Ash, %	6.0
Chitin, %	21.4
Crude protein, %	27.9
Calcium carbonate, %	40.0
Elements	
Ca, ppm	3.65
Co, ppm	< 0.01
Cu, ppm	2.30
Mn, ppm	0.29
Ni, ppm	0.20
Fe, ppm	2.93
Zn, ppm	1.83

Table 1.3 Analysis of shrimp heads and shells (Meyers, 1986).

	DM basis, %					
	Corrected protein[1]	Fat	Chitin	Ash	Ca	P
Shrimp heads	53.5	8.9	11.1	22.6	7.2	1.68
Shrimp shells	22.8	0.4	27.2	31.7	11.1	3.16

[1] Percentage of crude protein minus nitrogen associated with chitin.

1. Soil amendments and compost. In recent years, application of fresh shrimp waste to tillable soil has become more common. Generally, the waste is spread to a thickness of less than one inch and then tilled under immediately after application to minimize odor and insect problems (Hilderbrand, 1993). The nitrogen and mineral levels are beneficial amendments to the soil.

Composting shrimp waste has also become more common in coastal areas. The fresh waste is usually mixed in a 1:2 ratio with woodchips or other high carbon by-products, and turned daily, while composting for 60 to 90 days (Cato, 1992). The compost is then added as a soil amendment to tillable soil (Cato, 1992).

2. Shrimp meal. The technology exists for the drying and processing of shrimp waste into shrimp meal. However, the finished product is generally nutritionally inferior to fish meal, making it difficult to recover the processing costs. Meyers (1986) characterized the difference in protein levels of shrimp meal depending on the processing method (Table 1.4). Shrimp head meal is notably higher than whole carcass shrimp meal in the following amino acids: alanine, arginine, cystine, glycine, histidine, isoleucine, lysine, and valine (Meyers, 1986; Table 1.5).

Table 1.4 Protein and chitin levels of various shrimp meals (Meyers, 1986).

	DM basis, %		
Shrimp Meal	Crude protein	Chitin	Corrected protein[1]
Dehydrated	37.3	20.6	28.5
Sun-dried	51.7	9.0	47.8
Shrimp heads	58.2	11.1	53.5
Shrimp hulls	45.9	54.2	22.8

[1] Percentage of crude protein minus nitrogen associated with chitin.

3. Feedstuff for aquatic animals. Shrimp waste is very common in the diets of farmed shrimp in the U.S. and Far East (Meyers, 1986). Shrimp meal, as an ingredient, can comprise from 10 to 30% of the final diet formulation (Meyers, 1981). Researchers speculate that chitin plays an important role in crustacean metabolism (Meyers, 1981). Shrimp waste contains feeding stimulants, such as amino acids and nucleotides, which enhance its palatability for many aquatic species.

Shrimp waste can be an important natural source of carotenoids (Meyers, 1981), used to enhance flesh coloration in cultured trout and salmon, skin pigmentation in broiler chickens, and yolk color in eggs (Meyers, 1986). Carotenoids are degraded from excessive heat in the drying process, therefore, dried meals have a lower carotenoid content than fresh or frozen wastes (Meyers, 1986).

Table 1.5 Amino acid composition of shrimp meal (Meyers, 1986).[1]

Amino acid	Shrimp meal (sun-dried)	Shrimp head meal
Alanine	5.29	7.56
Arginine	6.31	6.70
Aspartic acid	10.74	9.07
Cystine[2]	1.59	2.35
Glutamic acid	14.56	13.61
Glycine	4.29	6.57
Histidine	1.90	2.23
Isoleucine	3.26	6.20
Leucine	7.57	6.72
Lysine	6.17	9.20
Methionine	2.84	1.65
Phenylalanine	4.56	4.60
Proline	3.44	3.31
Serine	4.53	3.49
Threonine	4.28	4.22
Tryptophan	1.26	0.63
Tyrosine	3.64	3.64
Valine	4.42	6.77

[1] Assumed to be percent of total amino acids.
[2] 50% cysteine.

4. Chitosan extract. Chitin, an N-acetyl glucosamine, is a straight chain polysaccharide which is similar in structure to cellulose (Ashford, 1977). A derivative of chitin is chitosan, which has several important industrial applications.

Uncharged amine groups have a high capacity to bind metal ions (Ashford, 1977). Some possible applications include the following: cationic flocculent in waste water treatment, cosmetics, animal feed, food additive, cholesterol reducing agent, and wound treatment (Skaugrud and Sargent, 1990). Ashford (1977) recommends that crustacean processors mechanically separate protein for use in animal feeds and sell remaining shell (chitin) waste as a raw material for chitin products. However, even after 20 years of commercial availability, the market for chitin is still very limited (Skaugrud and Sargent, 1990).

CRAB WASTE

Overview of Crab Waste Production

In 1986, crab was economically the fourth most important seafood product in the United States, with a total annual catch of 149,000 metric tons (Abazinge et al., 1994). In a typical year, Dungenous crabs (*Cancer magister*) are harvested on the Oregon coast from December 1 through August 15. December and January account for 80% of the harvest, with the catch decreasing throughout the remainder of the season. From 1970 to 1992, an average of 4,227 metric tons (Hilderbrand, 1993) were harvested yearly off the Oregon coast, with 60% being processed (Costa, 1978). Of that 60%, 85% was disposed of as waste. On a national level, the average crab landings from 1984 to 1988 was 168,022 metric tons. Eighty to 90% of the total harvest was disposed of as waste (Brinsfield, 1980; Abazinge et al., 1994; Ayangbile et al., 1987-88a; Samuels et al., 1982). Of the 5,318 metric tons of crab that were landed in Oregon in 1991-92, approximately 2,659 metric tons were disposed of as waste (Hilderbrand, 1993).

According to Bates (1992), fresh crabs are split, and the legs are removed. The backs and viscera are discarded as raw waste. The legs are boiled for 12 minutes, and then shaken to separate the meat from the shell. The leg shells and

remaining meat are then discarded as waste. Crab waste includes the uncooked back, viscera, and the cooked leg shells and meat left from processing.

Chemical Composition

The dry matter (DM) of crab waste varies from 40-45% (Abazinge et al., 1986b; Samuels et al., 1982). Crab waste has been reported to have the following chemical analysis: 44.1% CP, .18% acid detergent fiber (ADF), 34.5% ash, 1.76% P, .11% Mg, 14.2% Ca (Samuels et al., 1982). The chitin in crab waste varies from 12.7 - 13.6% (Ayangbile and Fontenot, 1988).

Disposal

As is the case with shrimp waste, the costs of dumping in landfills is becoming prohibitive as a means of disposal. Current alternative uses for crab waste are as follows:

1. soil amendments and compost;
2. crab meal;
3. feedstuff for aquatic animals; and
4. chitosan extract.

1. Soil amendment and compost. The majority of crab waste disposed of on the Oregon coast is applied to farmland as a soil amendment. Once applied, the waste is immediately tilled under to minimize odor and pests. As a seafood byproduct, crab waste is high in nitrogen and $CaCO_3$ (57.9%), (Costa, 1978), making it an excellent fertilizer and soil enhancer for the acidic soils of the Oregon coast and Willamette Valley (Kreag, 1973).

Composting crab waste has also become more common in coastal areas. As is the case with shrimp waste, crab waste is usually mixed with woodchips or other high

carbon by-products in a ratio of 1:3, and turned daily while composting for 60 to 90 days (Cato, 1992). The compost is then added as an amendment to tillable soil (Cato, 1992).

2. Crab (waste) meal. Technology exists for the drying and processing of crab waste into crab meal. Crab meal can be incorporated at low levels into the diets of ruminants. The finished product however is generally of poorer nutritional quality than fish meal, making it difficult to recover the processing costs. The small size of most crab processing plants limits further processing of this by-product into crab meal due to the capital investment required (Abazinge, 1986a). These two factors have contributed to the decrease in popularity of processing crab meal (Ayangbile et al., 1987-88a). Velez et al. (1991) reported the DM of crab meal as 93.9% with the following analysis on a DM basis: 36.4% CP, 13.3% ADF, 46.2% ash, 13.0% Ca, .78% Mg and 1.57% P. Husby et al. (1980) separated King crab (*Paralithodes camtschatica*) meal with a 40 mesh screen and analyzed the different fractions for proximate composition (Table 1.6). The >40 mesh crab meal had higher percentages of ash, ADF and Ca and a lower percentage of CP and ether extract (EE) than the <40 mesh meal.

Table 1.6 Proximate composition of King crab meals sieved through a 40 mesh screen (Husby, 1980b).

Item	< 40 mesh	> 40 mesh	Whole
Dry Matter, %	95.4	95.6	95.6
Crude protein, %	48.1	35.7	41.7
Ether extract, %	4.2	1.8	3.0
Ash, %	31.7	41.3	36.9
Acid detergent fiber, %	15.6	27.4	20.6
Calcium, %	7.9	10.3	9.0
Phosphorus, %	1.4	1.7	1.6

3. Feedstuff for aquatic animals. Crab waste is not used as extensively as shrimp waste as a feedstuff for aquatic animals. However, its high protein content does allow for minimal use in this area.

4. Chitosan. The same possibilities exist for producing chitosan from crab waste as for shrimp waste. However, even after 20 years of commercial availability, the market for chitin is still very limited (Skaugrud and Sargent, 1990).

LOW QUALITY FORAGES

Many low quality roughages are available in the United States. A low quality forage is one that has an energy value of < 60% Total Digestible Nutrient (TDN) and a CP of < 10% (DM basis). Examples of low-quality forages are grain straw, corn stover, and in the Pacific Northwest, grass seed straw. Most low quality forages are produced in conjunction with a higher value product, yet the disposal of these low-quality forages can be costly to the producer.

In Oregon, grass seed has been produced since the 1940's. The straw has historically been disposed of through open field burning. Since 1988, mounting public pressure over smoke pollution has severely decreased the burning of grass seed fields, with total elimination planned for 1998. As a means of disposal, grass seed straw is being baled and removed from the field. In 1991, 734,469 metric tons of grass seed straw were produced. Over 226,000 metric tons were compressed and exported to Japan for use in livestock feeds due to a shortage of roughage in the country. The rest has been disposed of mainly by burning or composting (Hartung, 1992).

A study on Oregon grass seed straw reported that three species of grass seed produced residue with notable nutritional value (Stamm, 1992): tall fescue (*Festuca arundinacea Schreb*), perennial ryegrass (*Lolium perenne L.*), and bentgrass (*Agrostus L.*).

Of these three species, tall fescue is available in the largest quantity (243,000 metric tons), however it is not recommended in the feeding of livestock if it contains high endophyte levels.

Bentgrass straw is produced in a much lower quantity (22,727 metric tons) which makes it less available. Perennial ryegrass straw is available in a large quantity (189,360 metric tons) and is commonly used as a livestock feed (Hartung, 1992; Stamm, 1992). The nutritive value of grass seed straw is approximately 45 - 55% TDN, 4 - 10% CP, 38 - 49% ADF, and 60 - 74% neutral detergent fiber (NDF) (Stamm, 1992). Perennial ryegrass straw in Oregon is harvested in July and August with a 1991 mean harvest date of July 22. Some is directly delivered to a marketplace; the majority is put into storage. The straw is primarily harvested in small bales (65 lbs), however, larger rectangular bales (1,200 lbs) and round bales (800 lbs) are also used. The cost of straw varies from $18 to $36/metric ton depending on the harvesting method, storage time and hauling distance (Hartung, 1992).

DAIRY HEIFERS

The rumen of a dairy heifer is capable of digesting fibrous material at approximately two months of age (Church, 1988), although silages should be limited until three months of age due to high moisture content causing low DM intake (Jurgens, 1993). Most dairy producers aim to have heifers freshen at 24 months of age. From three to 24 months of age, the average dairy heifer consumes about 4.2 metric tons of DM, or approximately 8.4 metric tons/year of total feed. If the shrimp or crab wastes were incorporated at 10% of the total ration dry matter, it would take 24,000 heifers to consume the wastes. There are approximately 30,000 dairy heifers in western Oregon which could conceivably utilize these seafood wastes. The nutritional requirements for Holstein heifers are listed in Table 1.7. Calcium is generally required at about one percent of the ration DM for growing dairy heifers.

Table 1.7 Summary of NRC (1989) requirements for Holstein heifers gaining .7 kg/day.

	Body weight, kg				
	100	200	300	400	500
Age, mo	3	7	11	15	20
DM intake, kg	2.82	4.68	6.66	8.92	11.63
NEm (Mcal/kg)	.96	.98	.93	.86	.78
NEg (Mcal/kg)	.51	.42	.36	.31	.27
TDN, %	70.21	67.09	64.11	60.99	58.04
CP, %	16.01	14.66	12.00	12.00	11.99

NE_m = Net energy of maintenance.
NE_g = Net energy of gain.

SEAFOOD WASTES AS A FEEDSTUFF FOR RUMINANTS

Ruminant animals are able to digest low-quality forages which are high in fiber (cellulose and hemicellulose). There is some evidence that chitin can also be utilized by ruminal microorganisms. Crab meal and shrimp meal consists of 12.9 and 7.6% chitin, respectively (DM basis) (Patton and Chandler, 1975). Average ruminal solubilities of crab meal, shrimp meal, and purified chitin were 17.4, 35.7, and 21.5%, respectively (Patton and Chandler, 1975). Chitin digestibility by young ruminants of crab meal ranged from 26 to 87% with an average of 66% (Patton et al., 1975). Work by Brundage et al. (1981) and White (1981) have also indirectly demonstrated that chitin in crab meal was used by ruminal microflora. Husby et al., (1980a) and LaFlamme (1988) recommend that a chitin-containing feedstuff should be introduced into a ruminant's diet gradually to allow adaptation of the ruminal microflora to the chitin.

Ruminant Feeding Trials

Well-preserved crustacean waste-straw silages have been successfully fed to beef steers, sheep, and calves. Feeding finishing steers a 30% DM crab waste-straw silage improved average daily gain and feed efficiency compared to steers fed a 30% DM orchard grass hay diet (Ayangbile et al., 1987-88a). A 50:50 crab waste to wheat straw (DM basis) silage with 20% molasses and a silage inoculant fed to sheep had a 57% DM digestibility (Abazinge et al., 1986a). Holstein bull calves showed no significant decline in growth when fed crab waste meal up to 21% of the basal diet (DM basis; Patton et al., 1975). After shrimp and crab wastes have been ensiled, the feed is palatable to the ruminants and free of obnoxious odors.

PRESERVATION

The rapid deterioration of seafood waste warrants a quick preservation to minimize protein breakdown and odor production. The odors are primarily caused by the decomposition of the protein and the production of trimethylamine, dimethylamine and monomethylamine. The odors can also be caused by histamine, tyramine, phenylethylamine, indole and other amines formed during protein putrefication (Dyer and Mounsey, 1945). Commonly used methods of preservation include the following: Drying and freezing; Salting; Treating with additives; and Ensiling.

Drying and Freezing

Either of these methods are effective at quickly preserving feedstuffs. However, the high cost associated with drying or freezing seafood wastes causes these methods to be impractical for producing a low cost livestock feedstuff.

Salting

Salting of meat and animal by-products has been used as a means of preserving high-protein wastes for many years. Salts help to preserve products through increased osmotic pressure and the inhibitory properties of the anion (Van Soest, 1992). The fishing industry of Ghana utilizes salt at 7 - 19% as a major method for curing fish (Nerquaye-Tetteh and Johnson, 1988). Salt is a low-cost feed additive which is currently used in most livestock operations.

Additives Used to Preserve Fresh Seafood Wastes

Several additives have been used to prevent deterioration of the fresh crab and shrimp waste. Crab waste has been treated with the following organic acids and bases: .75% propionic/.75% formic acid (1:1), .2% sodium hypochlorite (NaOCl), .4% hydrogen peroxide (H_2O_2), 1.5% acetic acid, or 1.5% sodium hydroxide (NaOH) (Ayangbile et al., 1987-88b).

It was found that a concentration of .75% propionic /.75% formic acid would preserve crab waste for up to 14 days, as determined by the pH and the trimethylamine (TMA) count. Trimethylamine is a volatile compound associated with the odors of deteriorating seafood, the higher the TMA count, the greater the odors and the higher the decomposition.

Torrissen et al. (1981/82) successfully preserved shrimp waste for 14 days with 4.8% (v/w) of 50% (v/v) sulfuric acid, 1.2% (v/w) of propionic acid and 100 mg/kg of the antioxidant butylhydroxytoluene. Abazinge et al. (1986b) compared the ability of 1.5% formaldehyde, .75% propionic/.75% formic acid, or 10% liquid molasses to preserve fresh crab waste. The molasses treatment had the highest volatile fatty acids and CP concentration, and the lowest pH compared to the other preservatives.

The cost of organic acid additives to crab and shrimp silage for preservation prior to transporting and ensiling was $163 - 408/metric ton of silage produced. This

would prove non-economical in producing a low-cost feedstuff for dairy heifers. Addition of molasses is a less expensive means of preserving wastes ($13.20 - $33.00/metric ton) which would have a higher consumer acceptance than the acids and warrants further investigation.

Ensiling

The process of ensiling utilizes fermentation as a low-cost means of preserving forage for livestock consumption. The ensiling process minimizes the total loss of nutrients from harvest through feedout and can offer increased feed handling efficiency for large herds (Mahanna, 1994).

Ensiling can be divided into four separate phases: aerobic phase, lag phase, anaerobic fermentation phase, and stable phase. Each phase and the possible problems associated with that phase will be discussed.

Aerobic phase. Oxygen trapped in the air spaces of the silage mass is consumed by plant respiration and aerobic microorganisms. If the silo is well sealed, little or no oxygen will infiltrate the silage and the aerobic phase will last about 24 hours (Pitt, 1990). Plant respiration uses DM, oxygen and plant sugars to produce carbon dioxide, water and heat (Muck, 1987). If the silage is not well sealed or if excess oxygen is trapped in the silage mass, then plant respiration will continue creating excess heat and reducing the energy level of the silage (Muck, 1987). To minimize plant respiration, the silo should be filled quickly, packed tightly, and the top should be sealed immediately after packing (Muck, 1987; Pitt, 1990; Muck and Bolsen, 1991).

Lag phase. The population of aerobic bacteria decreases as the oxygen in the silage is depleted. In the absence of oxygen, plant cell membranes begin to lyse and break down. This allows the cell juices to become a growth medium and the anaerobic bacteria population begins to increase. The lysis of cell membranes provides sugars

for bacteria to ferment, however, it also releases plant enzymes. The enzyme degradation of polysaccharides is beneficial as they provide additional sugars for fermentation. Proteolytic enzymes are also released which break down protein to non-protein nitrogen (NPN) forms which can cause a loss of true protein (Pitt, 1990; Muck and Bolsen, 1991). In addition, the lysis of cells can produce effluent in silages of less than 35% DM. Effluent is high in readily digestible nutrients and can represent a significant loss if not collected (Muck and Bolsen, 1991).

Anaerobic fermentation phase. The anaerobic bacteria, such as lactic acid bacteria (LAB) and Enterobacteriaceae, grow and multiply rapidly, increasing in numbers to about 1 billion per gram of forage (Pitt, 1990; Muck and Bolsen, 1991). These bacteria are very efficient at producing acids to lower the pH of the crop, which has remained fairly stable to this point. The end products of bacterial fermentation of plant sugars is primarily lactic acid, but some acetic acid, ethanol, carbon dioxide, and other minor products are produced. Homofermentative bacteria are preferred because they produce only lactic acid which can be further fermented in the rumen and are more beneficial from a nutritional perspective (Muck and Bolsen, 1991). The production of acids leads to a rapid decline in silage pH. When the pH reaches 3.8 - 5.0 the bacteria die out and the silage is considered to be stable. The lowered pH reduces the activity of proteolytic enzymes, sparing proteins, and stops the growth of other anaerobic bacteria. Finally, a drop in pH increases the rate of chemical hydrolysis of some polysaccharides, such as hemicellulose, and decreases the fiber content of the forage (Muck and Bolsen, 1991). The phase of active fermentation usually lasts about two weeks, but can continue for up to 2 months. Forages wetter than 35% DM tend to fit into the first category, whereas fermentation in silages with more than 55% DM is quite slow (Muck and Bolsen, 1991).

Stable phase. This phase is described as having little or no biological activity and lasts until the silo is opened and the silage comes into contact with oxygen. During feedout the face of the silo has unrestricted exposure to oxygen. Aerobic microorganisms can cause large losses of DM and nutritional value during feedout

(Muck and Bolsen, 1991). Generally the face of the silo should be fed in less than three days to minimize feedout losses (Muck, 1987; Pitt, 1990).

Profile of a stable corn or grass silage. When evaluating corn or grass silage there are some general guidelines to establish if it is a stable silage. Mahanna (1994) reports that a stable corn or grass silage should have a pH of 4.0 - 4.5 and should be in the 35 - 55% DM range. A stable silage should have the following levels of fermented acids on a DM basis: lactic 6 - 8%, propionic 0 - 1%, acetic < 2%, and butyric < 0.1%. The temperature of the silage should never be greater than 15 - 20°C above ambient temperature. Generally this silage should have a sweet acetic smell (Mahanna, 1994).

Mini Silos. On an experimental level, mini-silos have been used as a means of testing small quantities of various combinations of feedstuffs and additives (Ayangbile et al., 1987-88, Morrison, 1988; Samuels et al., 1991). Mini-silos vary in size from a 150 ml glass bottle (Morrison, 1988), to a 210 L barrel double-lined with polyethylene bags (Ayangbile et al., 1987-88b). Regardless of which silo type is used, it has been reported that the following factors must be considered: packing pressure, air tightness, repeatability, and an effluent trap. A separate silo must be used for each combination and desired sampling time. Once a silo has been sampled, its contents have been disrupted and fermentation changed, thus it cannot be further used as a fermentation test.

Mini-silos do not offer the field characteristics of heat flow and heat accumulation, hydrostatic pressure and packing, and possible anaerobiosis, in comparison to bunker and upright silos (Parker, 1978). Barnett (1954) suggests a three foot internal diameter and six foot internal height for best heat accumulation. It was reported that a silo with a one meter diameter is notably influenced by ambient temperature and recommended a 1.5 meter internal diameter (Parker, 1978). Mini-silos should be stored out of direct sunlight as it will affect heat of fermentation. Ensiling time periods have been reported to vary from 40 to 112 days (Parker, 1978).

Ensiling of shrimp and crab waste and low quality forage. There are many advantages to ensiling shrimp and crab wastes with low quality forages. Ensiling of seafood waste and low quality forages could produce a product that has an improved DM content and higher nutrient yield than either by-product ensiled individually. Ensiling is effective in destroying coliforms (Caswell et al., 1975), salmonella (Creger et al., 1973), and staphylococci (McCaskey and Anthony, 1975), but not Listeria (Pearson and Marth, 1990).

A low quality forage would serve as a sponge to absorb the high moisture crustacean waste and limit effluent, as well as increase the storage of the seasonally available product (Samuels et al., 1991). Increased DM content of the crustacean waste-straw silage would increase it's manageability and lower on-farm handling cost as compared to fish silage, which is currently handled in liquid form through the use of tanks and pumps.

A mixture of the wastes and straw could achieve a 40 - 55% DM that will be more conducive to proper fermentation (Muck, 1990; Samuels et al., 1991). The process of fermentation could also decrease the odor associated with crustacean waste and increase the palatability of both products (Samuels et al., 1991; Stephenson et al., 1991).

Seafood waste and straw are low cost by-products and once combined, could produce a low cost feedstuff for dairy heifers. Addition of a third product, possibly a cereal grain, or another by-product may be needed to increase the energy level of the feedstuff, provide substrate for fermentation, and increase the value of the final product (Samuels et al., 1991; Harrison et al., 1991).

Additives for ensiling crab and shrimp wastes and low quality roughages. A number of different additives have been used to improve the silage quality of crab or shrimp wastes when ensiled with low quality roughages. These include the addition of 0, 10, or 20% molasses (wet basis), 0 or 5.4% phosphoric acid, 1% formic acid, 1.5% propionic/formic acid, 16% glacial acetic acid or .1% microbial inoculant (*Streptococcus faecium* and *Lactobaccilus plantarum* blend (Samuels et al., 1982; Abazinge et al., 1986b; Ayangbile et al., 1987-88a). Most organic acid additives

have been shown to promote fermentation, however they also increase butyric acid production, which decreases the palatability of the final product to livestock. In addition, the organic acids are expensive, corrosive to farm equipment, and present health hazards to the operator. The addition of molasses maintains the good qualities of the organic acids without being caustic and leads to a decrease in the fermented silage pH, increased palatability and energy of the silage. Therefore, the addition of various concentrations of molasses at different dry matter levels will be investigated in this research project.

BIOTECHNOLOGY TRANSFER

The technology to ensile large quantities of seafood silage is available. Chopped grass straw and shrimp or crab wastes can be mixed in desired proportions and packed into plastic bag tunnels (Ag-Bag Corp., Warrengton, OR). For example, a bag which is eight feet in diameter and 100 feet long will hold 90-100 tons of silage.

Custom baggers could be hired to arrange delivery of grass straw, crustacean wastes and other ingredients and to ensile the feeds. The cost could be shared by the dairy producer and seafood industry. Bags could be conveniently located at heifer facilities. Seafood silage could be offered as part of a diet as grass silage or as a complete feed depending on the ingredients included in the silage.

METHODS OF DETECTING DETERIORATION IN SEAFOOD WASTES

There are three main methods for detecting deterioration in crab and shrimp waste. Two of the methods are subjective evaluations that include odor evaluation and inspection for microbial colonization. The other procedure, entailing a fair amount of technical expertise, is TMA analysis of volatile amines in seafood waste.

Samuels et al. (1982) used subjective odor evaluation as a characteristic of decomposition. The breakdown of seafood waste occurs rapidly and produces offensive amines at an increasing rate. A panel of at least four evaluators familiar with feedstuffs which are normally used is desired. Each panelist evaluates each treatment on a basis of whether or not it would be accepted by the animal when compared to other feedstuffs. Samuels et al. (1982) used a simple scale of either desirable or undesirable. Other scales could be used for a wider range of comparisons if a larger number of treatments are to be evaluated.

In an anaerobic environment, at higher pH's, mold growth could become established in different treatments of seafood waste. This mold growth will increase to break down the waste, cause unpalatability, and possibly even become toxic to animals. It is important to visibly inspect the ensiled waste for mold growth prior to disturbing the sample (Abazinge et al., 1986b). This inspection should be done by more than one individual, and if possible spot inspections under a microscope could detect early starts of the mold.

The TMA testing procedure was developed in 1945, and is commonly performed in most seafood labs. The TMA level provides the best indication of bacterial deterioration in seafood waste of any of the spoilage affects known. Dyer and Mounsey, (1945) reported that bacteria first utilize the lactic acid and sugars in the tissues of the seafood waste, thus reducing the TMA-oxide present in the waste to the odoriferous volatile base TMA. Trimethylamine production constitutes a warning of incipient spoilage by bacterial action. Dimethylamine (DMA) is a secondary spoilage product and can also be measured (Dyer and Mounsey, 1945). It has shown that at least 94% of the TMA produced comes from the reduction of TMA-oxide (Dyer and Mounsey, 1945).

Trimethylamine oxide (TMAO) is naturally present in many marine species and functions physiologically in a role similar to urea or uric acid in land animals. Trimethylamine oxide is excreted as a means of maintaining the nitrogen balance in the marine animal and levels are highest in sharks and teleosts (bony fish). Among the teleosts, the gadoid family (cod, pollock, haddock, whiting, hake and cusk) have the highest TMAO levels (Suyama and Suzuki, 1975).

The TMA assay was developed in 1945 by Dyer and Mounsey. This is a colorimetric method, fish is treated with trichloracetic acid (TCA) and formaldehyde solutions to extract TMA. This is followed by treatment to stabilize the volatile amine, the addition of picric acid working solution, and measurement of absorbance of the yellow picrate salt at 410 nm. The sample must be frozen prior to preparation for analysis to prevent any further decomposition. In 1957, Hashimoto and Okaichi found that the TMA method could be improved by the addition of KOH. This reduces formation of DMA and its interference with the TMA analysis.

A high pressure liquid chromatography (HPLC) method was developed for the measurement of TMA by Gill and Thompson (1984). This method involves extraction of TMA from the muscle with perchloric acid and subsequent chromatography of the PCA extract using sodium hydroxide as the elution solvent. From start to finish, this assay requires approximately 30 minutes. This method has two advantages over the following gas chromatograph (GC) method. The alkylamines will probably undergo less thermal decomposition on an HPLC column than on a GC column. It is also possible to analyze both volatile and nonvolatile compounds on the same column on the HPLC.

The GC method for determination of volatile amines in seafood was developed in 1987 (Perez Martin et al., 1987). The amines are extracted from the seafood by blending 20 g of seafood with TCA solution in a food blender and then filtering. The clear extract is then diluted to 100 ml with TCA. The amines are separated by using a precolumn of Chromosorb® 103 with a main column of Carbopack® coated with Carbowax® 20M and KOH (® = Registered trademark of Supelco, Inc., Bellfonte, PA). A flame ionization detector and a nitrogen-phosphorus-specific flame ionization detector can be used. Recovery of known amounts of TMA added to fish carcasses was around 86%, which was higher than either HPLC or the Dyer method.

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2. PRESERVING FRESH CRAB OR SHRIMP WASTE WITH MOLASSES OR SALT

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ABSTRACT

Five experiments were conducted to test preserving fresh shrimp or crab waste with molasses or salt. In Experiment 1, crab waste was combined with liquid molasses at 0, 5, 10, and 15% and stored in mini-silos for 14 days. The addition of molasses decreased pH, crude protein percentage and loss, and improved the odors. On day 14, the 15% molasses treatment had a crude protein of 26.6%, a crude protein loss from day 1 of .5 percentage points, a pH of 7.6, and a questionable odor score. In Experiment 2, fresh shrimp waste was combined with dry molasses at 0, 10, 15, 20, and 25%, and inoculant at either 0 or .1% and then ensiled for six days. Addition of molasses decreased the crude protein percentage and loss, ammonia concentration, pH, and acetic and butyric acid production while increasing dry matter percentage, dimethylamine and lactic acid concentrations and improving odors. The inoculated 25% molasses treatment had a crude protein of 25.0%, crude protein loss from day 1 of 0.68 percentage points, pH of 6.4, lactic acid of 16.19% and a questionable odor score. Experiment 3 was similar to Experiment 2 except shrimp waste at the 15 and 20% molasses levels was extended to 21 days to test stability. On day 21 the 20% molasses treatment had a crude protein of 25.1%, pH of 7.4, acetic acid of 3.78%, butyric acid of 4.11%, lactic acid of .14%, and a poor odor score.

For both Experiment 4 and 5, shrimp or crab waste was combined with salt at 0, 2.5, 5.0, 7.5, 10.0, and 12.5%. The addition of salt decreased crude protein percentage and loss, ammonia concentration, and all volatile fatty acids while

increasing the pH and improving the odors in both wastes. Shrimp waste with salt at 7.5% had a crude protein of 29.9%, crude protein loss of 2.8 percentage points, a pH of 8.3 and a good odor score. Crab waste at 7.5% salt had a crude protein of 30.7%, crude protein loss of 2.7 percentage points, pH of 8.5, and a good odor score. Given the need to quickly preserve the rapidly deteriorating seafood waste, treatment with salt was more successful at preserving crude protein and minimizing odor production than either dry or liquid molasses.

INTRODUCTION

Recent economic incentives in Oregon have lured seafood plants from offshore to onshore processing. This has created a large quantity of seafood wastes for which there is no readily available method of disposal. In Oregon, there is an estimated 1,045 metric tons of crab waste and 6,364 metric tons of shrimp waste processed annually (Hilderbrand, 1993). These wastes, ranging from 30 to 40% crude protein (CP), are high in moisture and extremely perishable. Past work has shown that fresh crab waste can be successfully ensiled with molasses and wheat straw (Abazinge et al., 1994). The small quantity processed daily on the Oregon coast warrants stockpiling the waste for 5 to 7 days to produce a quantity sufficient for farm scale ensiling. The perishability of these wastes make it necessary to first preserve them prior to ensiling. An alternate preservation method is to use salt which increases the osmotic pressure in the wastes due to the inhibitory properties of the anion (Van Soest, 1992). The goal of these experiments was to economically preserve fresh shrimp or crab waste which would be conducive to subsequent ensiling.

MATERIALS AND METHODS

Fresh shrimp or crab wastes were collected from Newport Shrimp (Newport, OR) within five hours after processing and used in five experiments designed to preserve the wastes.

Experiment 1. Ensiling Crab Waste with Liquid Molasses

The raw crab (whole shells and viscera) was processed through a meat grinder to a maximum particle size of 1 cm, resulting in a slurry mixture. The processed raw (backs and viscera) and cooked (legs and meat) crab waste were mixed 50:50 to produce a crab waste blend. In this 4 X 2 factorial arrangement, the liquid molasses was added to the crab wastes at 0, 5, 10, and 15% (wt./wt. basis) and mixed together in a Uebler Mixing Cart (Uebler Manufacturing, Vernon, NY) and then stored in 15 L mini-silos with or without a lid (factor 2). Thermocouples (Omega Engineering, Inc. Stamford, CT) were inserted into the mini-silos with lids which were then stored in an unheated building during February. Room and thermocouple temperatures were recorded at 0700 and 1900 daily. Wastes were sampled on day 0 and then designated mini-silos were opened on day 1, 3, 7, and 14 post-ensiling. The waste was sampled and the mini-silo then removed from the study. Within 1 hr of sampling, pH was recorded and samples were frozen for future analysis. Samples were later dried, ground, and analyzed for dry matter (DM), CP, ether extract (EE) (Stoldt, 1952), ash (AOAC, 1980), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Robertson and Van Soest, 1981). Extremely varied results were recorded from ashing the waste at incrementally higher temperatures greater than 550°C. Therefore ash percentages are only reported for the fresh shrimp and crab waste and not for individual treatments. A total of 32 treatments in duplicate, or 64 mini-silos were used for this experiment.

Experiment 2. Preservation of Shrimp Waste with Dry Molasses

Dry sugarcane molasses (42% sugars) was mixed with the shrimp waste at levels of 0, 10, 15, 20, and 25% (wt./wt. basis) in this 5 X 2 factorial arrangement. A mixed bacterial inoculant (BioPower Silage Inoculant, Bio-Techniques, Redmond WA) was added to the mixes at a level of 0 or 0.1% (factor 2). The inoculation rate was 1.0 X 10^8 colony forming units (cfu's) per gram of wet shrimp waste (i.e. 0.1% equals 1 gram of inoculant per kg of shrimp waste). Thirty-two kg of wet shrimp waste were put into the mixer (reversible bottom auger) and dry molasses was added at correct proportions [i.e. 10% = 3.2 kg of molasses (32 X .10)]. The 3.2 g of inoculant was hydrated in 320 ml of water then added slowly to the mixing shrimp waste to help facilitate even inoculation. After mixing for two min., four samples were taken from the homogeneous mixture. One sample was analyzed within one hr for pH and ammonia concentration while the remaining samples were labeled and immediately frozen at -20°C. Shrimp waste was tightly compacted into plastic lined 15 L mini-silos and sealed. Thermocouples were inserted into the center of the wastes and an air seal was installed. Odor evaluations of good, questionable, or poor were made by a panel of three persons on day 0 and day 6. The mini-silos were opened on day 6, homogenized in a Hobart mixer and four samples were collected. One sample was analyzed within 1 hr for pH and ammonia concentration; the three remaining samples were labeled and immediately frozen (-20°C). Waste samples were diluted 1 to 25 with distilled water and the concentration of ammonia measured with an ion electrode (Meter 720A; Orion 9512, Boston, MA). All frozen samples were later analyzed for DM, CP, lactic acid and volatile fatty acids (VFA's) (Fussell and McCalley, 1987), as well as trimethylamines (TMA's) and dimethylamines (DMA's). Sample preparation for TMA and DMA analysis was similar to the lactic acid and VFA preparation (Fussell and McCalley, 1987) except for the replacement of 2 ml of .12 M oxalic acid with 2 ml of distilled water. Trimethylamine and DMA concentrations were determined by gas chromatography (Hewlett Packard, Wilmington, DE, HP 5890 Series II with automatic sampler 7673 and flame

ionization detector). A carbopack B/4% CW 20 M/0.8% KOH (2 m X 2 mm ID; Supelco, Inc., Bellfonte, PA) with a nitrogen carrier flow of 18 ml/min. at 70°C was used. The detector and injector temperatures were both 180°C. Room temperature, maximum and minimum room temperatures, and mini-silo temperatures were recorded at 0900 daily. A total of 10 treatments in triplicate, or 30 mini-silos were used for this experiment.

Experiment 3. Molasses Preservation of Shrimp Waste for 21 Days

Dry sugarcane molasses (42% sugars) was added to the shrimp waste at 15% and 20% (wt./wt. basis) in an incomplete 2 X 2 factorial arrangement. A mixed bacterial inoculant (BioPower Silage Inoculant, Bio-Techniques, Redmond WA) was added to the mixes at a level of 0 or 0.1% (factor 2). The same procedures were followed as in Experiment 2 except these mini-silos were sampled on day 21. A total of 4 treatments in triplicate, or 12 mini-silos were used for this experiment.

Experiment 4 and 5. Shrimp-Salt and Crab-Salt Preservation

Two subsequent experiments were conducted to test salt (NaCl) as a preservative. Salt was added to the wet shrimp or ground crab wastes at levels of 0, 2.5, 5, 7.5, 10, and 12.5 (wt./wt. basis) in two five-level comparison experiments. Twenty-four kg of waste were put into the mixer (reversible bottom auger) and the desired amount of salt was added to reach the designated concentration [i.e. 5% = 1.2 kg of salt (24 X .05)]. The same procedures for mixing, sampling, and analysis were followed as in Experiment 2 and lids were placed on the buckets but not sealed. A total of 12 treatments in triplicate or 36 mini-silos were used for these experiments.

Statistical Analyses

Statistical analyses were performed using analyses of variance by the GLM procedures of SAS (1988). The model included treatment main effects and interactions between main effects. For Experiment 1, all main effects and interactions with a P value $\leq .05$ will be considered significantly different. For all other experiments a P-value of $< .01$ will denote significantly difference. Means were compared using LSD and considered significantly different if $P < .01$.

The natural logarithm of ammonia concentrations, ppm, was used after a preliminary analysis of untransformed data showed evidence of heterogeneity of residuals. Logarithms of homogeneous transformed data are reported in text and tables.

RESULTS AND DISCUSSION

Fresh crab waste used in Experiments 1 and 5 had the following composition (mean ± standard error): DM, % 37.5 ± .8; CP, % 32.3 ± .4; ADF, % 22.8 ± 2.5; ash, % (550°C for 6 hours) 45.6 ± .5; and a pH of 8.4 ± .1. Fresh shrimp waste used in Experiments 2, 3 and 4 had the following composition: DM, % 25.2 ± 2.2; CP, % 41.9 ± .6; ADF, % 28.3 ± .1; ash, % (550°C for 6 hours) 35.2 ± .5 and a pH of 9.5 ± .1.

Experiment 1. Preservation of Crab Waste with Liquid Molasses

The purpose of testing the preserving crab waste with or without a lid was to determine the facility necessary to store the waste at the processing plant. No differences were found between lid treatments except for temperature, therefore data presented is the means of both treatments within the molasses level.

Results of crab waste analyses on day 14 post-ensiling are reported in Table 2.1. As the level of molasses increased from 0 to 15%, DM percentage significantly increased (35.5 to 39.5%, respectively). The DM of liquid molasses is 72% which accounts for the increase in DM with the addition of molasses.

The percentage of CP was similar, ranging from 27.4 to 25.5% with the addition of 0 to 15% molasses. As molasses level increased, ADF, NDF, and EE gradually decreased (Table 2.1). These decreases are most likely attributed to a dilution effect of the added liquid molasses.

Starting CP percentages for 0, 5, 10, and 15% molasses approximated from the fresh shrimp analysis were 32.0, 30.3, 28.7, and 27.1%, respectively. The loss in CP is highest for 0% molasses and decreases numerically as the level of molasses increases (Table 2.1). The CP loss is least (0.5 percentage points) at the 15% molasses level and is significantly different from losses for 0, 5, and 10% molasses

treatments indicating more CP is preserved and a higher quality end-product is produced at the 15% molasses level. When Abazinge et al. (1986) added 10% molasses to crab waste, they found an increase in CP in the 10% molasses treatment over the control. This can either be attributed to the preserving nature of the sugars in the molasses or the fermentation of increased carbohydrates to produce acids and partially preserve the wastes. These acids would be driven off in the drying process and consequently the CP percentage would increase.

The pH of the crab waste with the addition of any molasses was significantly lower than the control (Table 2.1). However, a pH of 7.6 is still too high to be considered an adequate endpoint for lactic acid fermentation (Mahanna, 1994).

The mean temperature of all mini-silos was 10.2°C compared to a mean room temperature of 5.6°C. Figure 2.1A depicts ensiling temperature over time and illustrates that ensiling temperature was parallel to room temperatures. The effect of ambient temperature on the ensiling temperature is related to the mini-silo size (Parker, 1978). These data indicate that the ensiling temperature of the 15 L mini-silos used in this experiment is greatly affected by room temperature. The mini-silos with lids averaged 8.4°C compared to the mini-silos without lids which averaged 12.1°C. Mini-silos without lids were consistently higher than the sealed mini-silos (Figure 2.1B). The aerobic environment in the mini-silos without lids allowed for rapid decomposition of the wastes and the subsequent increase in temperature.

The addition of 5% molasses or greater produced a silage with questionable odor evaluation compared to 0% molasses which was evaluated as poor (Table 2.1). Abazinge et al. (1986) found 10% molasses to decrease the odor production in crab waste. The decrease in odor production can be attributed to either the preserving action of the sugars or the ability of the sweet odor of the molasses to mask the unpleasant odor of the crab waste. It was concluded that crab waste with molasses from 0 to 15% and with or without a lid was not adequate in preserving fresh crab waste quickly enough to prevent deterioration and odors. Molasses at higher levels will be tested on shrimp waste to determine if a more adequate fermentation would occur.

TABLE 2.1 Chemical analyses of crab waste on day 14 post-ensiling at four levels of liquid molasses (Experiment 1)[1].

	Molasses, %					
Item	0	5	10	15	SEM	P-value
DM, %	35.5[a]	37.6[b]	38.9[b,c]	39.5[c]	.4	.0006
CP, %[2]	27.4	26.6	25.5	26.6	.6	.1420
CP, loss[2,3]	4.6[a]	3.7[a]	3.2[a]	.5[b]	.6	.0117
ADF, %[2]	16.7[a]	16.7[a]	16.1[a]	14.0[b]	.3	.0064
NDF, %[2]	26.9[a]	24.3[b]	23.7[b]	20.4[c]	1.0	.0361
EE, %[2]	3.8[a]	3.0[b,c]	3.2[b]	2.7[c]	.2	.0087
pH	8.1[a]	7.8[b]	7.6[b]	7.6[b]	.1	.0170
Odor[4]	3.0[a]	1.5[b]	1.8[b]	1.5[b]	.3	<.0001

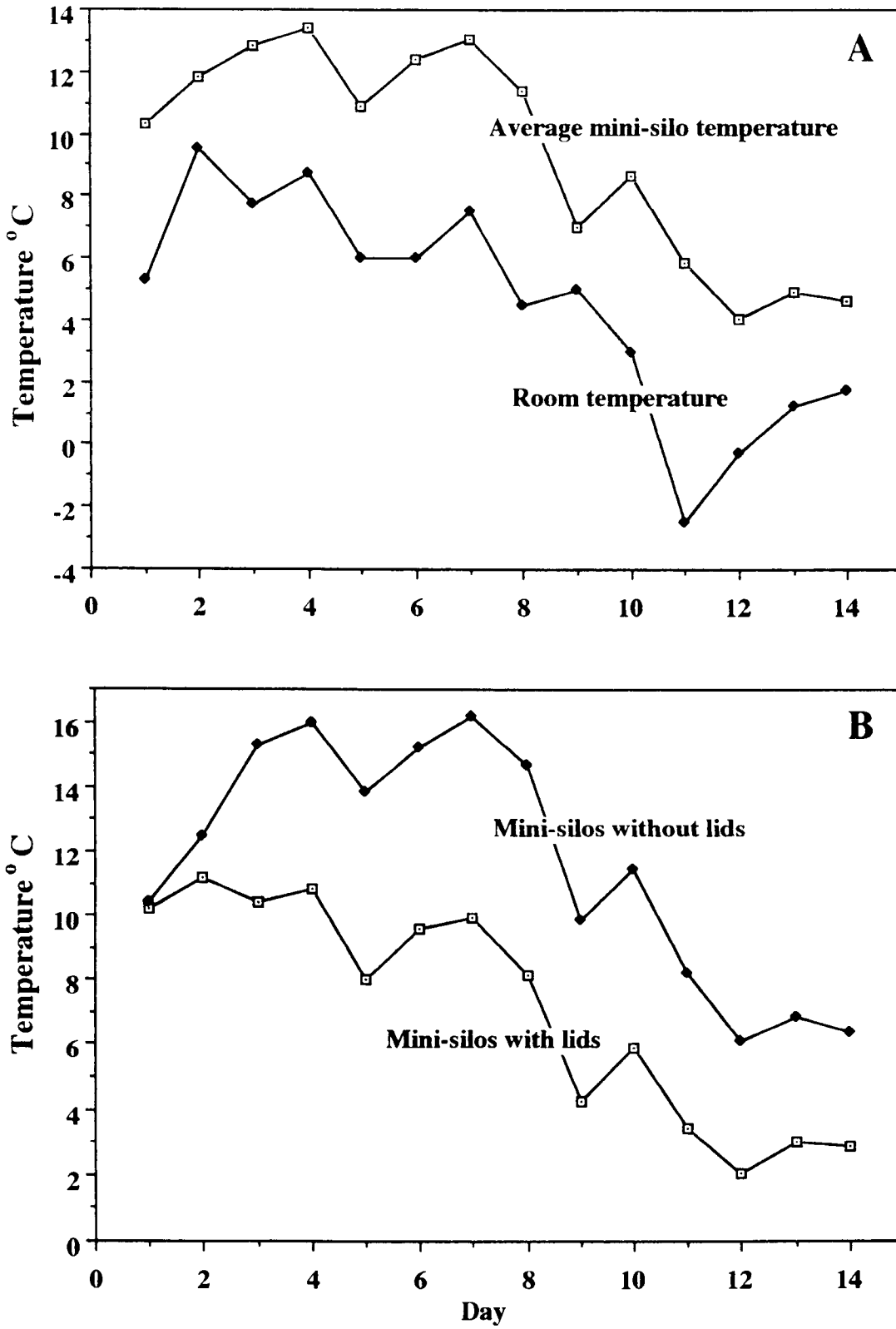
[a,b,c] Means in same row with different superscript differ (P≤.05).
[1] Least square means and standard error of mean (SEM).
[2] DM basis.
[3] CP loss = CP on day 0 minus CP on day 14 in percentage units.
[4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

Experiment 2. Preservation of Shrimp Waste with Dry Molasses

DM and CP changes. Fresh shrimp waste had a mean DM of 27.9% and the DM of dry molasses was 92%. As the level of molasses increased from 0 to 25%, DM percentage significantly increased from 26.6 to 42.0%, respectively (Table 2.2). The increase in DM is due in part to the addition of molasses, but can also be attributed to the variability of DM in the fresh shrimp waste. Because water collected in the bottom of the containers, the fresh shrimp varied in DM based on its location in the 115 L transport containers.

As inoculant level increased from 0 to .1%, the DM increased significantly from 33.7 to 35.6 (Table 2.2). A significant molasses by inoculant interaction occurred (Table 2.3). At 0, 10, and 20% molasses, DM's were similar within inoculant treatments. At 15 and 25% molasses, the DM of the inoculated shrimp waste was significantly higher than the non-inoculated shrimp waste (Table 2.3).

Figure 2.1 Temperature of crab waste preserved with molasses.
Panel A. Average mini-silo temperature vs. room temperature.
Panel B. Mini-silos with lids vs. mini-silos without lids.



On day 0, the CP of fresh shrimp waste and dry molasses was 41.9 and 9.3%, respectively. As the level of molasses increased from 0 to 25%, CP percentage decreased from 34.6 to 25.8%, respectively (Table 2.2). This can be attributed to a dilution effect with the addition of molasses. A significant molasses by inoculant interaction occurred. At 0 and 15% molasses, CP was significantly lower for inoculated shrimp waste than for non-inoculated shrimp waste; at 10, 20, and 25% molasses CP was similar within inoculant treatments (Table 2.3). On day 0, both the inoculated 0 and 15% molasses treatments were much lower than the non-inoculated treatments in CP. This accounts for most of these interactions which is best explained by the CP difference.

The CP difference (day 0 minus day 6) decreased significantly from 4.6 to -1.9 percentage units as the level of molasses increased from 0 to 25% (Table 2.2). This apparent increase in CP may be attributed to the loss of DM when structural carbohydrates were fermented to produce acids (Mahanna, 1994). In the drying process, the acids were evaporated and therefore the CP became more concentrated. A significant molasses by inoculant interaction occurred. Addition of inoculant decreased CP loss at all molasses levels except the 15% molasses level.

The ammonia concentration in fresh shrimp waste was 22.2 ppm (natural log 3.1 ppm). As the level of molasses increased from 0 to 25% the ammonia [log] concentration decreased from 8.3 to 4.7 ppm respectively, (Table 2.2). A significant molasses by inoculant interaction occurred. At 15 and 20% molasses levels, ammonia concentrations were significantly lower for inoculated shrimp waste than non-inoculated shrimp waste; at 0, 10, and 25% molasses ammonia concentrations were similar within inoculant treatments (Table 2.3). It is possible that at molasses levels 0 to 20% carbohydrates are in short supply. At 0 and 10% molasses, the carbohydrates are depleted by a wide variety of bacteria early in the fermentation process and are unable to minimize ammonia production. At 15 and 20% molasses, the inoculant increases the lactic acid producing bacteria population such that they are able to utilize most of the molasses and decrease proteolysis and ammonia concentration.

The level of DMA in fresh shrimp waste was 126 ppm on day 0. The DMA concentration increased significantly with the addition of 10 and 20% molasses compared to the 0% molasses treatment (Table 2.2). At 15% and 25% molasses the DMA concentrations were similar to the 0% molasses treatment. The DMA concentrations were similar within both inoculant treatments (Table 2.2). The level of TMA in fresh shrimp waste was 109 ppm on day 0. The TMA concentrations were similar within all molasses and inoculant treatments with an overall mean of 312.1 ppm (Table 2.2). This data indicates that treatment with molasses was unsuccessful in reducing the bacterial reduction of trimethylamine oxide to produce trimethylamine (Dyer, 1945).

Ensiling Parameters. The pH of the fresh shrimp waste with molasses at 0, 10, 15, 20, and 25% was 9.5, 8.7, 8.5, 8.7, and 8.0, respectively. As molasses increased from 0 to 25%, pH decreased from 7.7 to 6.8 respectively (Table 2.2). A significant molasses by inoculant interaction occurred. At 25% molasses, the pH was significantly lower for the non-inoculated shrimp waste (6.4) than for inoculated shrimp waste (7.2) (Table 2.3). The pH for 0, 10, 15, and 20% molasses was similar within inoculant treatments. On day 0, the pH of the 25% molasses inoculated treatment was 8.2 and the non-inoculated treatment was 7.8. This lower starting pH for the non-inoculated shrimp waste is probably the explanation for the day 6 difference in pH between treatments. The acetic acid concentration of fresh shrimp waste was 0.13% on day 0. As molasses increased from 0 to 25%, acetic acid decreased from 3.24 to 1.20% and as inoculant increased from 0 to 0.01%, acetic acid decreased from 2.19 to 1.82 (Table 2.2). Both molasses and inoculant promote a lactic acid fermentation, therefore under normal conditions, increasing amounts of either of these additives should decrease the amount of acetic acid produced in favor of the stronger lactic acid (Mahanna, 1994). A significant molasses by inoculant interaction occurred. At 0 and 15% molasses, acetic acid was significantly lower for inoculated shrimp waste than for non-inoculated shrimp waste (Table 2.3). At 10, 20, and 25% molasses acetic acid was similar within inoculant treatments.

The level of propionic acid in fresh shrimp waste was .06 on day 0. As molasses increased from 0 to 10% and from 20 to 25%, propionic acid was unchanged. At 15% molasses, proprionic acid increased to .58%, and then decreased to .05% at 20% molasses. As inoculant increased from 0 to .01% propionic acid decreased from .27 to .18% (Table 2.2). A significant molasses by inoculant interaction occurred. Propionic acid levels were similar for all levels of molasses except 15%, in which it was significantly lower for the inoculated shrimp waste than the non-inoculated shrimp waste.

The level of butyric acid in fresh shrimp waste was .02% on day 0. As molasses increased from 0 to 25% butyric acid decreased from .33 to .01% and as inoculant increased from 0 to .01% butyric acid decreased from .24 to .15%. At 0 and 15% molasses, butyric acid was significantly lower for inoculated shrimp waste than non-inoculated shrimp waste (Table 2.2). Butyric acid was significantly higher for the inoculated shrimp waste than non-inoculated shrimp waste at 10% molasses. Butyric acid concentrations for 20 and 25% molasses were similar within inoculant treatments.

The lactic acid concentration of fresh shrimp waste was 1.03% on day 0. As molasses increased from 0 to 25% lactic acid increased from .38 to 14.34% (Table 2.2). A molasses by inoculant interaction occurred. At 15% molasses lactic acid was significantly higher for inoculated shrimp waste than non-inoculated shrimp waste, whereas at 25% molasses, the inoculated shrimp waste was significantly lower than the non-inoculated shrimp waste (Table 2.3). At 0, 10, and 20% molasses, lactic acid concentrations were similar within inoculant treatments.

Concentrations of isobutyric, isovaleric and valeric acids were .01, .04, and .00% in the fresh shrimp waste on day 0. As the level of molasses increased from 0 to 25%, isobutyric, isovaleric, and valeric acids decreased significantly on day 6 (Table 2.2). As inoculant increased from 0 to .01%, isobutyric acid decreased from .06 to .04% (Table 2). A molasses by inoculant interaction occurred with isobutyric and isovaleric acids. Isobutyric and isovaleric at 0% molasses were significantly lower for the inoculated shrimp waste than the non-inoculated shrimp waste (Table

TABLE 2.2 Main effects of dry molasses and inoculant on chemical analyses of shrimp waste on day 6 post-ensiling (Experiment 2).

	Molasses, %							Inoculant, %			
	0	10	15	20	25			0	.01		
Item						SEM	P-val			SEM	P-val
DM, %	26.6[a]	33.8[b]	34.8[b]	36.2[b]	42.0[c]	.6	<.0001	33.7	35.6	.4	.0035
CP, %[1]	34.6[a]	33.3[b]	31.2[c]	28.4[d]	25.8[e]	.3	<.0001	31.0	30.3	.2	.0261
CP, dif[2]	4.6[a]	2.4[b]	-.7[c]	-.8[c,d]	-1.9[d]	.3	<.0001	1.5	.0	.2	<.0001
NH_3[3,4]	8.3[a]	7.1[b]	6.2[c]	5.0[d]	4.7[e]	.1	<.0001	6.5	6.1	0.0	<.0001
DMA[4]	138[a,c]	215[b]	168[a,b]	211[b]	123[a]	14	.0002	163	178	9	.2188
TMA[4]	217	347	294	404	299	62	.3215	292	332	39	.4689
pH	7.7[a]	7.4[b]	7.5[a,b]	7.3[b]	6.8[c]	.1	<.0001	7.3	7.4	0.0	.0107
ACET, %[1]	3.24[a]	2.26[b]	1.96[c]	1.36[d]	1.20[d]	.07	<.0001	2.19	1.82	.05	<.0001
PROP, %[1]	.24[a]	.19[a,c]	.58[b]	.05[c]	.10[a,c]	.04	<.0001	.27	.18	.03	.0301
ISOB, %[1]	.19[a]	.04[b]	.02[b]	.00[b,c]	.00[c]	.01	<.0001	.06	.04	.00	.0021
BUT, %[1]	.33[a]	.29[a]	.31[a]	.02[b]	.01[b]	.02	<.0001	.24	.15	.01	.0002
ISOV, %[1]	.13[a]	.00[b]	.00[b]	.00[b]	.00[b]	.01	<.0001	.03	.02	.00	.0201
LACT, %[1]	.38[a]	7.17[b]	9.24[c]	13.84[d]	14.34[d]	.34	<.0001	8.94	9.04	.21	.7515
VAL, %[1]	.14[a]	.08[a,b]	.04[b]	.00[b]	.00[b]	.02	.0005	.05	.06	.01	.8306
Odor[5]	3.0[a]	3.0[a]	2.5[b]	2.0[c]	1.8[c]	.1	<.0001	2.5	2.5	.1	1.000

[a,b,c,d,e] Means within treatment in same row with different superscripts differ ($P < .01$).
[1] DM basis. [2] CP difference (day 0 minus day 6) in percentage points. [3] Values for NH_3 (ammonia) are natural logarithms of original data.
[4] Measurements in ppm. [5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 2.3 Interactions of dry molasses and inoculant on chemical analyses of shrimp waste on day 6 post-ensiling (Experiment 2).

	Molasses, %											
	0		10		15		20		25			
	Inoculant, %											
	0	.01	0	.01	0	.01	0	.01	0	.01		
Item											SEM	P-val
DM, %	26.8[a]	26.4[a]	35.1[b,c]	32.5[c]	33.0[c]	36.6[b]	36.5[b]	35.9[b,c]	37.4[b]	46.6[d]	.9	<.0001
CP, %[1]	35.9[a]	33.4[b]	33.0[b]	33.5[b]	32.2[b]	30.1[d]	28.7[d,e]	28.2[e,g]	25.0[f]	26.5[f,g]	.4	.0004
CP, dif[2]	6.03[a]	3.18[b,c]	3.31[b]	1.51[c,e]	-1.23[d]	-.19[d]	-.08[d,e]	-1.54[d,f]	-.68[d]	-3.14[f]	.42	.0014
NH_3[3,4]	8.2[a]	8.4[a]	7.2[b]	7.0[b,c]	6.8[c]	5.7[d]	5.6[d]	4.4[e]	4.7[e,f]	4.8[f]	.1	<.0001
DMA[4]	125	152	211	218	150	187	222	200	109	137	20	.5850
TMA[4]	173	261	410	283	236	352	440	367	199	399	87	.3327
pH	7.8[a]	7.6[a,b]	7.5[a,b,c]	7.3[b,c]	7.4[b,c,e]	7.7[a,e]	7.4[b,c,e]	7.2[c]	6.4[d]	7.2[c]	.1	<.0001
ACET, %[1]	3.50[a]	2.99[b]	2.25[c]	2.28[c]	2.45[c]	1.46[d]	1.42[d]	1.31[d]	1.36[d]	1.05[d]	.10	.0007
PROP, %[1]	.22[a]	.25[a]	.11[a]	.26[a]	.94[b]	.21[a]	.07[a]	.03[a]	.04[a]	.15[a]	.06	<.0001
ISOB, %[1]	.22[a]	.15[b]	.03[c,d,e]	.05[c]	.03[c,e]	.01[d,e]	.01[d,e]	.00[d]	.00[d]	.00[d]	.01	<.0001
BUT, %[1]	.42[a]	.25[b]	.17[b,c]	.41[a]	.55[d]	.06[c,e]	.03[e]	.01[e]	.00[e]	.01[e]	.03	<.0001
ISOV, %[1]	.15[a]	.10[b]	.01[c]	.00[c]	.00[c]	.00[c]	.00[c]	.00[c]	.00[c]	.00[c]	.01	.0075
LACT, %[1]	.40[a]	.37[a]	6.54[b]	7.80[b]	7.90[b]	10.59[c]	13.70[d,f]	13.98[d,f]	16.19[e]	12.48[e,f]	.48	<.0001
VAL, %[1]	.17	.12	.02	.14	.08	.00	.00	.00	.00	.00	.03	.0350
Odor[5]	3.0	3.0	3.0	3.0	2.5	2.5	2.0	2.0	1.8	1.8	.2	1.000

[a,b,c,d,e,f,g] Means in same row with different superscripts differ (P < .01).
[1] DM basis. [2] CP difference (day 0 minus day 6) in percentage units. [3] Values for NH_3 are natural logarithms of original data.
[4] Measurements in ppm. [5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

2.3). Concentrations of isobutyric and isovaleric acids at 10, 15, 20, and 25% molasses were similar between inoculant treatments.

In summary, the three major acids for characterizing ensiling are acetic, butyric, and lactic (Mahanna, 1994). As the level of molasses increased, acetic and butyric decreased while lactic increased. This indicates that the increasing levels of molasses promoted a lactic acid fermentation. Also at 15% molasses, the inoculated shrimp waste had lower acetic and butyric and higher lactic acid suggesting that the 15% molasses level created an environment in which inoculation was beneficial at enhancing the ensiling process.

As molasses increased from 0 to 25%, the odors of the shrimp waste moved from poor into the questionable range (Table 2.2). As the level of molasses increased from 0 to 25% the average daily mini-silo temperatures increased from 19.1 to 20.5°C. The overall average mini-silo temperature for all treatments during the 6 days was 19.9°C compared to an average room temperature, maximum, and minimum of 17.9, 24.4, and 14.6°C, respectively. The elevated mini-silo temperatures indicates that some degree of microbial or bacterial activity was taking place. It appears that a lactic acid fermentation had began in the molasses treatment of 15, 20 and 25%. However, the use of 25% molasses would be costly. Because this experiment only lasted for 6 days, the following experiment was designed to allow 21 days for fermentation to progress to test the practicality of using molasses as a preservative.

Experiment 3. Molasses Preservation of Shrimp Waste for 21 Days

CP changes. The starting CP for the 15 and 20% molasses levels were 27.7 and 25.5% on day 0. The CP loss was significantly lower on day 21 for the 20% molasses treatment than the 15% molasses treatment (Table 2.4). Treating shrimp waste with inoculant also significantly lowered the CP loss at both molasses levels.

TABLE 2.4 Main effects of dry molasses and inoculant on chemical analyses of shrimp waste on day 21 post-ensiling (Experiment 3).

	Molasses, %			Inoculant, %			
	15	20		0	.01		
Item			P-val			SEM	P-val
DM, %	27.0	26.9	.9081	26.7	27.2	1.0	.7618
CP, %[1]	24.9	25.1	.5147	24.8	25.2	.2	.0964
CP, dif[2]	2.7	.4	<.0001	2.0	1.1	.2	.0062
NH_3[3]	3676	3561	.3627	3535	3701	84	.2008
DMA[3]	116	164	.0258	134	146	12	.5245
TMA[3]	1238	457	.1846	614	1082	381	.4100
pH	7.5	7.4	.0747	7.5	7.4	0	.0623
ACET, %[1]	5.43	3.78	.0002	4.74	4.48	.18	.3275
PROP, %[1]	4.09	3.27	<.0001	3.79	3.58	.08	.0923
ISOB, %[1]	.12	.05	.0008	.07	.10	.01	.0693
BUT, %[1]	2.57	4.11	<.0001	2.94	3.74	.13	.0020
ISOV, %[1]	.06	.01	.2288	.05	.02	.03	.4274
LACT, %[1]	.12	.14	.8813	.09	.17	.06	.3791
VAL, %[1]	.35	.30	.2765	.25	.40	.03	.0073
Odor[4]	1.7	2.7	.0007	2.3	2.0	.1	.1114

[a,b] Means within treatment in same row with different superscripts differ (P < .01).
[1] DM basis.
[2] CP difference (day 0 minus day 6) in percentage points.
[3] Measurements in ppm.
[4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

The ammonia concentration of the inoculated 15% molasses treatment was significantly higher than the non-inoculated shrimp waste where a molasses by inoculation interaction occurred (Table 2.5).

Ensiling parameters. There was a molasses by inoculant interaction for pH. The pH for the inoculated 15% molasses treatment was significantly lower than the non-

inoculated shrimp waste (Table 2.5). The pH of the inoculated 20% molasses treatment was significantly higher than the non-inoculated shrimp waste.

Acetic, propionic, and isobutyric acid concentrations decreased as the molasses level increased (Table 2.4). Butyric acid concentrations increased as the level of molasses increased from 15 to 20% and as inoculant increased from 0 to .01% (Table 2.4). A molasses by inoculant interaction occurred for propionic acid. The inoculated 15% molasses treatment was significantly lower in propionic acid than the non-inoculated shrimp waste (Table 2.5). Whereas the inoculated 20% molasses treatment was significantly higher than the non-inoculated shrimp waste.

There was significantly lower odor production from the 15% molasses treatment (questionable) when compared to the 20% molasses treatment (poor) (Table 2.4).

The mini-silos in this experiment had an overall average temperature of 20.2°C compared to an average room temperature, maximum, and minimum of 21.9, 30.5, and 13.0, respectively. Compiling the data from the 6 day trial (Experiment 2) and the 21 day trial (Experiment 3) allows for some interesting conclusions. In Experiment 2, a lactic acid fermentation had began for the 15 and 20% molasses treatments as is indicated by the pH and acid concentrations. In Experiment 3, the concentrations of the acids would suggest that a poor bacterial fermentation took place and that deterioration was predominant by day 21. If it is assumed that the first 6 days of Experiment 3 paralleled Experiment 2, and that a lactic acid fermentation actually began, then it can be hypothesized that fermentation ended before reaching a desired pH range. It is possible that *Candida* and *Hansenula* yeast metabolized lactic acid to form acetic and butyric acid. This resulted in an elevated pH and led to mold growth and deterioration of the ensiled shrimp waste (Mahanna, 1994). The three previous experiments proved molasses unsuccessful at quickly preserving crab or shrimp waste, therefore preservation with salt will be attempted.

TABLE 2.5 Interactions of two levels of dry molasses and inoculant on chemical analyses of shrimp waste on day 21 post-ensiling (Experiment 3).

	Molasses, %, Inoculant, %					
	15, 0	15, .01	20, 0	20, .01		
Item					SEM	P-val
DM, %	27.5	26.6	26.0	27.7	1.4	.3558
CP, %[1]	25.0	24.9	24.6	25.6	.3	.0648
CP, dif[2]	3.2	2.2	.83	.0	.3	.7940
NH_3[3]	3370[a]	3981[b]	3700[a,b]	3421[a,b]	119	.0057
DMA[3]	154	174	115	118	17	.6411
TMA[3]	985	1492	243	671	538	.9437
pH	7.7[a]	7.4[b]	7.4[b]	7.5[c]	0	<.0001
ACET, %[1]	5.97	4.89	3.50	4.06	.25	.0112
PROP, %[1]	4.76[a]	3.42[b]	2.81[c]	3.73[b]	.11	<.0001
ISOB, %[1]	.11	.12	.04	.07	.01	.6192
BUT, %[1]	1.97	3.16	3.90	4.32	.18	.0626
ISOV, %[1]	.09	.02	.01	.01	.04	.4053
LACT, %[1]	.10	.14	.07	.20	.09	.6638
VAL, %[1]	.21	.49	.29	.32	.04	.0211
Odor[4]	1.8	1.5	2.8	2.5	.2	1.000

[a,b,c] Means within treatment in same row with different superscripts differ (P < .01).
[1] DM basis.
[2] CP difference (day 0 minus day 6) in percentage points.
[3] Measurements in ppm.
[4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

Experiment 4 and 5. Salt Preservation of Shrimp and Crab Wastes

Results of salt preservation of shrimp and crab waste are reported in Tables 2.6 and 2.7, respectively. As the level of salt increased from 0 to 12.5%, the DM percentage increased significantly from 21.6 to 31.1% in shrimp waste and from 38.8 to 44.4% in crab waste. As the salt level increases from 0 to 12.5%, the CP of

shrimp and crab waste decreases from 35.9 to 26.7% and 31.4 to 25.1% respectively. This can be attributed to a dilution of the CP with the addition of salt.

The CP difference decreases significantly in the shrimp waste as the level of salt increased, with salt over 7.5% yielding less than one percentage point loss of CP (Table 2.6). Although not statistically different, the CP difference in crab waste also tended to decrease as salt level increased (Table 2.7).

The ammonia concentrations decreased significantly in both shrimp and crab waste from 3641 to 60 ppm and from 1212 to 90 ppm, respectively (Table 2.6 and 2.7). The lowered ammonia concentrations indicate that protein degradation has decreased.

In crab waste, DMA significantly increased from 105 to 725 ppm as the salt level increased from 0 to 12.5% (Table 2.7). The shrimp waste had notably lower concentrations of both TMA and DMA than the crab waste on day 7 (Table 2.6). The starting TMA and DMA for crab waste were 1664 $\pm$ 730 and 420 $\pm$ 212 ppm, respectively. These high starting concentrations indicate that crab waste was rapidly deteriorating when delivered to the lab and explains the extremely high TMA and DMA concentrations on day 7.

The pH of both salt treated wastes increased as the level of salt increased (Table 2.6 and 2.7). In shrimp waste the pH remained similar at 0, 2.5, and 5.0% salt and then increased at the 7.5 and 10.0% levels. Where as the pH of crab waste increased at the 2.5 and 5.0% salt levels but then remained similar at the 7.5, 10.0, and 12.5% salt levels.

In shrimp waste, the concentration of all VFA's decreased significantly as the level of salt increased from 0 to 12.5% (Table 2.6). This indicates that as the level of salt increased most bacterial and microbial activity decreased. Acetic acid concentration was the highest of all VFA's at 3.5% at the 0% salt level but decreased quickly to .20% at the 7.5% salt level.

In crab waste the concentrations of acetic and propionic acid, both which were lower at 0% salt than shrimp waste, decreased as the salt level increased (Table 2.7). All other VFA's were non-detectable in the salt treated crab waste.

Odor evaluation of both shrimp and crab waste were identical with the 0 and 2.5% salt being poor, 5% being questionable, and all levels greater than 5% salt being rated to have a good odor (Table 2.6 and 2.7).

TABLE 2.6 Analyses of shrimp waste on day 6 post-treatment at six levels of salt (Experiment 4).

	Salt, %							
	0	2.5	5.0	7.5	10.0	12.5		
Item							SEM	P-val
DM, %	21.6[a]	21.1[a]	22.7[a]	28.9[b]	28.2[b]	31.1[c]	.4	<.0001
CP, %[1]	35.9[a]	33.9[b]	32.1[b]	29.9[c]	26.6[d]	26.7[d]	.4	<.0001
CP, dif[2]	6.0[a]	3.0[b]	5.4[a]	2.8[b]	.7[c]	.1[c]	.4	<.0001
NH_3[3,4]	8.2[a]	7.3[b]	6.8[c]	5.3[d]	4.8[e]	4.1[f]	.1	<.0001
DMA[4]	125	161	199	147	190	171	18	.0925
TMA[4]	173	202	243	165	197	205	41	.7994
pH	7.8[a]	7.5[a]	7.6[a]	8.3[b]	8.8[c]	9.0[c]	.1	<.0001
ACET, %[1]	3.5[a]	3.05[b]	1.79[c]	.20[d]	.08[d]	.06[d]	.09	<.0001
PROP, %[1]	.22[a]	.11[b]	.07[b,c]	.01[d]	.04[c,d]	.05[c]	.01	<.0001
ISOB, %[1]	.22[a]	.03[b]	.02[b]	.00[b]	.00[b]	.00[b]	.01	<.0001
BUT, %[1]	.42[a]	.02[b]	.03[b]	.00[b]	.01[b]	.01[b]	.01	<.0001
ISOV, %[1]	.15[a]	.05[b]	.05[b]	.00[b]	.00[b]	.00[b]	.00	<.0001
LACT, %[1]	.41	.82	.47	.05	.25	.25	.19	.1813
VAL, %[1]	.17[a]	.01[b]	.01[b]	.00[b]	.00[b]	.01[b]	.03	.0033
Odor[5]	3.0[a]	3.0[a]	2.0[b]	1.0[c]	1.0[c]	1.0[c]	0	<.0001

[a,b,c,d,e,f] Means in same row with different superscripts differ (P < .01).
[1] DM basis.
[2] CP difference (day 0 minus day 6) in percentage points.
[3] Values for NH_3 are natural logarithms of original data.
[4] Measurements in ppm.
[5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

The level of salt had no affect on bucket temperature in either shrimp or crab waste. The overall bucket temperature in shrimp waste was 19.4°C compared to an average room temperature, maximum and minimum of 17.0, 24.8, and 15.7°C, respectively. Although there was no difference between salt treatments, the 2.4°C increase in bucket temperature over room temperature indicates that some form of microbial or bacterial action was taking place with at least some of the treatments. The crab waste treatments had an overall bucket temperature of 6.7°C compared to an average room temperature, maximum, and minimum of 7.0, 11.7, and 6.1, respectively.

At levels of 7.5% and greater, salt was successful at minimizing or stopping microbial breakdown in shrimp waste. Salt at 5% and greater decreased CP loss and lowered the ammonia concentrations in crab waste.

TABLE 2.7 Analyses of crab waste on day 6 post-treatment at six levels of salt (Experiment 5).

	Salt, %							
	0	2.5	5.0	7.5	10.0	12.5		
Item							SEM	P-val
DM, %	38.6[a]	41.3[b]	43.3[c]	44.4[d]	43.9[c,d]	44.4[d]	.2	<.0001
CP, %[1]	31.4	31.7	29.7	30.7	27.2	25.1[b]	1.6	.0691
CP, dif[2]	.86	1.96	.79	-2.66	.13	-1.38	1.6	.3938
NH_3[3,4]	7.1[a]	6.5[b]	4.7[c]	4.3[d]	4.4[d]	4.5[c,d]	.1	<.0001
DMA[4]	105[a]	140[a]	996[b]	744[b]	834[b]	725[b]	93	<.0001
TMA[4]	820	1136	2118	2029	2201	2161	327	.0375
pH	7.2[a]	7.5[b]	8.5[c]	8.5[c]	8.5[c]	8.5[c]	.1	<.0001
ACET, %[1]	.66[a]	.22[b]	.02[c]	.01[c]	.01[c]	.01[c]	.01	<.0001
PROP, %[1]	.02[a]	.00[b]	.00[b]	.00[b]	.00[b]	.00[b]	.00	<.0001
LACT, %[1]	.24[a]	.13[b]	.17[b]	.15[b]	.14[b]	.13[b]	.02	.0242
Odor	3.0[a]	3.0[a]	2.0[b]	1.0[c]	1.0[c]	1.0[c]	0	<.0001

[a,b,c,d] Means in same row with different superscripts differ (P < .01).
[1] DM basis.
[2] CP difference (day 0 minus day 6) in percentage points.
[3] Values for NH_3 are natural logarithms of original data.
[4] Measurements in ppm.
[5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

CONCLUSION

Protein degradation and ammonia production occur rapidly in seafood waste products making speed of preservation vital to maintaining the level of protein in the fresh wastes. During Experiments 1, 2, and 3, a lactic acid fermentation of the wastes was attempted. There were indications that fermentation began, but ended before reaching the desired pH range and a stable product. This can be attributed to either the rapid deterioration of the wastes, lack of fermentable carbohydrates, the high buffering capacity of the wastes, or a combination of these factors. Molasses at greater than 25% may decrease the pH in the waste, however this would become cost prohibitory and the fermentation process would still take at least 6 days. In an attempt for a quicker, lower cost preservation, Experiments 4 and 5 were conducted to test salt as a preservative. Salt, at a 7.5% level or greater, in both wastes, produced a product with good odor, minimal ammonia production, and a high level of preserved CP. This was a low cost method to preserve and store crustacean waste which minimized degradation and odor production.

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3. ENSILING SALT-PRESERVED SHRIMP OR CRAB WASTE WITH GRASS STRAW, MOLASSES AND INOCULANT

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ABSTRACT

Two experiments were conducted to test the ensiling characteristics of salt-preserved shrimp or crab waste. A third experiment was conducted to test the ruminal degradability of fresh shrimp and crab waste and shrimp and crab silage. In Experiment 1, shrimp waste, preserved with 7.5% salt and stored for 6 days, was ensiled with molasses at 0, 10, or 20%, inoculant at 0 or 0.01%, and perennial rye grass straw to achieve 37, 47, or 52% dry matter. Treatments were stored in 15 L mini-silos for 40 days. As the dry matter percentage increased, crude protein, acid detergent insoluble nitrogen, percent acid detergent insoluble nitrogen, ether extract percentage, ammonia and acetic, propionic, butyric and lactic acid concentrations, salt content, and volume of effluents significantly decreased, while acid detergent fiber percentage increased and pH, dimethylamine and trimethylamine concentrations and odors remained similar. As molasses level increased crude protein, acid detergent insoluble nitrogen and lactic acid concentration increased while pH and acid detergent fiber, ammonia, acetic, propionic, butyric and isovaleric acid concentrations decreased, percent acid detergent insoluble nitrogen, dry matter, dimethylamine and trimethylamine concentrations remaining similar and odors improved. The addition of inoculant decreased percent acid detergent insoluble nitrogen, pH, butyric and isovaleric acid concentration and improved odors. High quality shrimp silages were produced at 47% dry matter at 10 or 20% molasses and at 52% dry matter at 10%

molasses. The 47% dry matter, 20% molasses silage had a crude protein of 16.1% and an acid detergent fiber of 27.0% on a dry matter basis, a pH of 7.0 and a good odor evaluation.

Experiment 2 was similar to Experiment 1 except that salt-preserved crab waste was used and perennial rye grass straw was added to achieve 43, 46, or 52% dry matter. As dry matter percentage increased, crude protein, ether extract, dimethylamine, and trimethylamine concentrations also increased while acid detergent fiber, acid detergent insoluble nitrogen, percent acid detergent insoluble nitrogen, ammonia, and lactic acid concentration and effluents decreased; odors remained the same. As molasses increased, dry matter, ether extract, dimethylamine and lactic acid concentration also increased while crude protein, acid detergent fiber, acid detergent insoluble nitrogen, percent acid detergent insoluble nitrogen, acetic acid and salt concentration and pH decreased and odors improved. The addition of inoculant decreased dry matter, acid detergent insoluble nitrogen and percent acid detergent insoluble nitrogen content. The data indicates that a lactic acid fermentation took place and good quality silages were produced at 46% dry matter with 10 or 20% molasses and 52% dry matter at 10% molasses. The 46% dry matter, 10% molasses, inoculated silage had a crude protein of 21.1% and an acid detergent fiber of 19.6% on a dry matter basis, a pH of 6.6 and a good odor evaluation.

In Experiment 3, two fistulated steers were used to test the ruminal degradability of fresh shrimp and crab waste and shrimp and crab silage at 9 time points ranging from 0 to 96 hr. Shrimp waste had a ruminal availability of dry matter, crude protein, acid detergent fiber, and acid detergent insoluble nitrogen of 36.9, 43.0, 44.2, and 43.6 % respectively. Crab waste had a ruminal availability dry matter, crude protein, acid detergent fiber, and acid detergent insoluble nitrogen of 38.9, 53.3, 41.0, and 33.0 % respectively. Ensiling the shrimp and crab wastes improved the percentage of ruminal availability dry matter and crude protein and lowered the percentage of ruminal availability of acid detergent fiber and acid detergent insoluble nitrogen. This indicates that ensiling promotes the degradation of chitin and the release of nitrogen to the rumen.

INTRODUCTION

Recent economic incentives in Oregon have lured seafood plants from offshore to on-shore processing. This has created a large quantity of seafood waste for which there is no readily available method of disposal. These wastes, ranging from 30 to 40% crude protein (CP), are high in moisture and extremely perishable. Previous work in this laboratory has shown that fresh shrimp and crab wastes can be quickly preserved with 7.5% salt.

Chitin, in crab meal, has been shown to have an average digestibility in young ruminants of 66% (Patton and Chandler, 1975). Work by Brundage et al. (1981) and White (1981) have also indirectly demonstrated that chitin in crab meal was used by ruminal microflora. It is possible that the process of ensiling may have an affect on the digestibility of chitin in crab or shrimp waste. The objectives of these experiments include 1) to ensile the salt-preserved shrimp and crab wastes with grass seed straw, molasses and inoculant; 2) to determine if a lactic acid fermentation took place; and 3) to measure the dry matter (DM), CP, acid detergent fiber (ADF), and acid detergent insoluble nitrogen (ADIN) disappearance of crab and shrimp waste in the rumen and compare it to crab and shrimp silage.

MATERIALS AND METHODS

Fresh shrimp and crab wastes were collected from Newport Shrimp (Newport, OR) within five hours of processing. The crab waste was ground and then both wastes were mixed individually with 7.5% salt and stored for 6 days (Chapter 2). The salt-preserved wastes were then used in the following two experiments.

Experiment 1. Ensiling Salt-Preserved Shrimp Waste with Grass Straw, Molasses and Inoculant

A 3 X 3 X 2 factorial design was used for ensiling the salt-preserved shrimp waste. The first factor being the addition of perennial rye grass straw (92% DM, 7.8% CP) to achieve three levels of dry matter, 37, 47, and 52%. The second factor was the addition of dry molasses (42% sugars) at 0, 10, and 20%. The third factor was the addition of mixed bacterial inoculant (BioPower Silage Inoculant, Bio-Techniques, Redmond WA) at 0 or .01%. This allowed for an inoculation rate of 1.0 X 10^7 colony forming units (cfu's) per gram of wet shrimp waste (i.e. .01% equals .1 gram of inoculant per kg of shrimp waste). The correct proportions of shrimp waste and grass straw were added to determined molasses and inoculant levels in the mixer (reversible bottom auger) to achieve the designated dry matter levels. After mixing for 2 min., four samples were collected from the homogeneous mixture. One was analyzed within 1 hr for pH and ammonia concentration, the other three were frozen immediately at -20°C. The remaining shrimp waste was equally divided into three separate 15 L buckets. Shrimp waste was tightly compacted into plastic lined mini-silos until full and then sealed. Thermocouples (Omega Engineering, Inc. Stamford, CT) were inserted and an air seal was installed. Room temperature and mini-silo thermocouples were read between 7:30 and 9:00 a.m. daily. Daily maximum and minimum room temperatures were recorded. The mini-silos, three per treatment, were opened on day 40, homogenized in a Hobart mixer, and four samples were collected. One sample was analyzed within one hr for pH and ammonia

concentration. Silage samples were diluted 1 to 25 with distilled water and the concentration of ammonia measured with an ion electrode (Meter 720A; Orion 9512, Boston, MA), the other three were frozen and later analyzed for DM, CP, ADF (Robertson and Van Soest, 1981), EE (Stoldt, 1952), %ADIN and ADIN (Goering and Van Soest, 1970; Goering et al., 1972), ash (AOAC, 1980), lactic acid and volatile fatty acids (VFA's) (Fussell and McCalley, 1987), dimethylamines (DMA's) and trimethylamines (TMA's) and NaCl titration. Sample preparation for DMA and TMA analysis was similar to the lactic acid and VFA preparation (Fussell and McCalley, 1987) except for the replacement of the 2 ml of .12 M oxalic acid with 2 ml of distilled water. The DMA and TMA concentrations were determined by gas chromatography (Hewlett Packard, Wilmington, DE, HP5890 Series 2 with automatic sampler 7673 and flame ionization detector). A Carpopack® B/4% CW 20 m/0.8% KOH (2 m x 2 mm inside diameter; ® = registered trademark of Supelco, Inc., Bellfonte, PA) with a nitrogen carrier flow of 18 ml/min. at 70°C was used. The detector and injector temperatures were both 180°C. The NaCl concentration was measured in the filtrate of the VFA extracts with a chloride titrator (Quantab Titrators, Chloride, Environmental test systems, Inc., Elkhardt, IN). A total of 18 treatments in triplicate or 56 mini-silos were used for this experiment.

One additional modified bucket per treatment was used for monitoring and measuring effluent. The effluent bucket was filled at the same time as each individual treatment was ensiled. Effluent was collected daily, starting on day 1 and every day thereafter until no measurable effluent existed for five consecutive days. The quantity of effluent was measured and recorded as well as three effluent samples taken for each bucket having effluent. One sample was tested within one hr for pH and a second sample on selected treatments was tested for biochemical oxygen demand (BOD; APHA, 1989) shortly after collection. The third sample was frozen directly after collection (-20°C) and was later analyzed for DM and CP.

Experiment 2. Ensiling Salt-Preserved Crab Waste with Grass Straw, Molasses and Inoculant

This experiment follows the same design, protocol and analyses as the previous experiment with the exception of different DM levels. Crab waste was ensiled at 43, 46, and 52% DM. To incorporate enough straw into the mixture to achieve a consistency of normal silage, it became necessary to add water to all of the 43% DM treatments and some of the 46% DM treatments.

Experiment 3. Ruminal Degradation of Shrimp and Crab Waste and Shrimp and Crab Silage

Crab or shrimp waste, which contain chitin, was placed into the rumen of two mature fistulated steers to allow adaptation of the ruminal microflora to chitin. The steers were stuffed daily with .5% of their body weight on a DM basis (approximately 2.7 kg) for two weeks, prior to the experiment. The stuffing was continued daily with the final stuffing at the same time the 24 hr samples were inserted. The steer diet consisted of 5 lbs of ground wheat and grass hay ad libitum. Four samples were tested: fresh shrimp waste, fresh crab waste, shrimp silage and crab silage. The shrimp silage used was the 47% DM, 20% molasses non-inoculated treatment from the Experiment 1 (Table 3.3). The crab silage used was the 46% DM, 10% molasses, inoculated treatment from the Experiment 2 (Table 3.7). All samples were dried (55°C for 24 hrs) and ground through a 5 mm screen. A 4 g sample for each feedstuff was put into a nylon 10 x 20 cm^2 bag with 53 μm pore size (Ankom Company, Fairport, NY), and then heat sealed. The bags were presoaked in water for five minutes, and then placed in the rumen in duplicate for 96, 72, 48, 24, 12, 9, 6, 3 and 0 hours with two blank bags for each time point (Nocek, 1988). All bags were removed and placed in ice water to arrest microbial action at 0 hrs. Bags were washed (de Boer et al., 1986), dried at 55°C for 24 hrs, and then analyzed for DM, CP, ADF, and ADIN. Percentage of DM, CP, ADF, and ADIN disappearance were analyzed as previously described. The DM, CP, ADF, and ADIN degradability data

(Table 3.9) were fitted by multivariate secant nonlinear iterative procedure of SAS (1988) as described by Shaver et al. (1986).

Statistical Analyses

Statistical analyses were performed using analyses of variance by the GLM procedures of SAS (1988). The model included treatment main effects and interactions between main effects. For all experiments a P-value of $< .01$ denoted statistical difference. Means were compared using LSD and considered significantly different if $P < .01$.

The natural logarithm of ammonia concentration, ppm, was used after a preliminary analysis of untransformed data showed evidence of heterogeneity of residuals. Logarithms of the homogeneous transformed data are reported in text and tables.

RESULTS AND DISCUSSION

Experiment 1. Ensiling Salt-Preserved Shrimp Waste with Grass Straw, Molasses and Inoculant

DM and CP changes. Analysis of main effects of DM, molasses and inoculant on shrimp silage on day 40 yielded a high F-value and a significant ($P < .0001$) DM effect for most variables. Therefore, the data was analyzed separately at each of the three DM levels.

Shrimp silages with initial DM of 37.1, 47.3, and 52.3% had a decrease in DM on day 40 of 2.9, 3.8, and 1.7 percentage points respectively (Table 3.1). These three DM levels were chosen as they cover the range of normal ensiling DM levels. The 52% DM is on the high end of the DM range as the literature recommends for ensiling feedstuffs of animal origin (Nilsson and Rydin, 1965).

At 37 and 47% DM, silage DM decreased by 2.6 and 1.2 percentage points, respectively (Table 3.2 and 3.3), as molasses level increased from 0 to 20%. There was a significant molasses by inoculant interaction at 52% DM. At 10% molasses, the addition of inoculant decreased DM by 4.0 percentage points, whereas at 0 and 20% molasses, the addition of inoculant had no affect on DM (Table 3.4). Under normal conditions, ensiling a waste of 50% DM or greater would have a DM loss of 8 - 10% (Harrison and Fransen, 1991). The mini-silo weight change was very small, therefore a 4.0 percentage point change in DM was approximately an 8% change in DM. This is an indication that a normal silage fermentation took place.

As DM increased from 37 to 52%, CP decreased from 18.8 to 11.5% (Table 3.1). This decrease can be attributed to a dilution of CP by changing proportions of grass straw:waste required to raise the DM level. As the level of molasses increased from 0 to 20%, the CP increased significantly from 14.0 to 15.8%. At 47 and 52% DM, a significant molasses by inoculant interaction exists. At 47% DM, the addition of inoculant at 0 and 10% molasses had no affect on CP%, while at 20% molasses the addition of inoculant significantly decreased CP by 1.3 percentage points (Table

3.3). At 52% DM, the addition of inoculant at 0% molasses significantly increased CP percentage while at 10% molasses, the addition of inoculant decreased CP percentage; and inoculant had no affect at the 20% molasses level (Table 3.4). Generally the addition of an inoculant increases the lactic acid bacteria population and results in less acetic acid, more lactic acid and a lower pH compared to the same silage without an inoculant. An inoculant can also reduce proteolysis and the ammonia fraction (Muck and Bolsen, 1991). As molasses increases, the effect of inoculant should also increase, however varied results were obtained from this experiment.

As DM increased from 37 to 52%, ADF also increased significantly from 21.3 to 34.9%. As molasses increased from 0 to 20%, ADF decreased significantly from 32.2 to 52.2% (Table 3.1). As DM increased from 37 to 52%, ADIN decreased from .57 to .25% (Table 3.1). Chitin, which makes up nearly 15% of crab and shrimp waste, can be measured by use of the ADIN procedures. This measures the nitrogen in the ADF fraction. The percentage of chitin, which contains nitrogen, can be estimated from the ADIN results. As the DM increases, and the ratio of waste to straw decreases, the ADIN would be expected to decrease as well. There was a significant molasses by inoculant interaction at all three DM levels. At 37% DM, the addition of inoculant significantly decreased ADIN at 0% molasses, increased ADIN at 10% molasses but failed to have an affect at the 20% molasses level (Table 3.2). At 47% DM, the addition of inoculant at 20% molasses decreased ADIN, but had no effect at 0 and 10% molasses (Table 3.3). At 52% DM, the addition of inoculant had no affect on ADIN at 0% molasses, decreased ADIN at 10% molasses, and increased ADIN at 20% molasses (Table 3.4).

As DM increased from 37 to 52%, percent ADIN decreased from 19.0 to 13.7% (Table 3.1). With the addition of inoculant, ADIN decreased from 16.6 to 15.8%. There was a significant molasses by inoculant interaction at 37 and 47% DM. At 37% DM, the addition of inoculant at 0% molasses decreased percentage of ADIN by 3.9 units, but failed to have an effect at the 10 and 20% molasses levels (Table 3.2).

As DM increased from 37 to 52%, EE decreased from 1.8 to 1.4% (Table 3.1). As the level of grass straw, which is low in ether extract, increases, it is expected that the EE of that treatment would decrease.

As DM increased from 37 to 52%, DMA and TMA increased by 123 and 121 ppm respectively. As the level of molasses increased from 0 to 20%, DMA significantly increased by 106 ppm while TMA decreased numerically by 75 ppm (Table 3.1). There was a significant molasses by inoculant interaction at 52% DM. The addition of inoculant at the 10% molasses level significantly reduced the DMA and TMA concentrations by 218 and 423 ppm respectively, while at the 0 and 20% molasses level the addition of inoculant failed to have an effect (Table 3.4). The non-inoculated 10% molasses shrimp silage at 52% DM had a DMA and TMA concentration of 482 and 773 ppm respectively. These high values indicate that the shrimp waste has continued to deteriorate during ensiling. However, the inoculated 10% molasses shrimp silage had lower DMA and TMA concentrations which were similar to the starting DMA and TMA levels.

Ensiling Variables. The pH of the 47% DM silage was 7.2. The pH was significantly lower for both the 37 and 52% DM silage at 6.8 and 6.9 respectively. As the level of molasses increased from 0 to 20%, the pH decreased significantly from 7.4 to 6.7. The addition of inoculant decreased the pH from 7.1 to 6.9. There was a significant molasses by inoculant interaction at 47% DM. At 0% molasses, the pH decreases with the addition of inoculant, but failed to do so at the 10 and 20% molasses levels.

Acetic and butyric acid concentrations decrease as the level of DM and molasses increased. The addition of inoculant significantly increased the butyric acid concentration. There was a significant molasses by inoculant interaction for acetic acid at the 52% DM level and butyric acid at the 47% DM level. Acetic acid concentration increased with the addition of inoculant at 0% molasses, but the addition of inoculant failed to have an effect at 10 and 20% molasses. The addition of inoculant increased butyric acid concentration at 0% molasses, decreased butyric acid concentration at 10%, and failed to have an effect at 20% molasses.

Lactic acid concentration decreased significantly as DM% increased and increased significantly as the level of molasses increased. Increasing levels of soluble carbohydrates, under normal conditions, increases production of lactic acid (Muck, 1987).

Although of less significance to the ensiling process, propionic, isobutyric, isovaleric and valeric tended to either decrease or stay unchanged as the level of DM increased and as the level of molasses increased.

Odor evaluation scores improved significantly as the level of molasses increased and as inoculant was added. There was a significant molasses by inoculant interaction at 37% DM. At 0% molasses at all three DM levels, the silages were scored to have poor odor with or without the addition of inoculant. At 10% molasses and 37% DM, the addition of inoculant moved the silage from a good odor score to a questionable one, but failed to have an effect at 47 or 52% DM. At 20% molasses, all silages received a good odor score except the inoculated 37% DM silage. The improvement in odor scores which parallels the increase in molasses, can be attributed to either a more complete fermentation or to the ability of molasses to partially preserve, and mask the odors of the seafood wastes.

The average room temperature, maximum, and minimum temperatures were 12.3, 24.1, and 9.4°C, respectively. The mini-silo temperature for the 37, 47, and 52% DM silages were 13.8, 14.7, and 13.8°C, respectively. It is known that the effect of ambient temperature on the ensiling temperature is related to the mini-silo size (Parker, 1978). Given the small size (15 L) of these mini-silos, the slight increase in mini-silo temperatures, over room temperature, at all three DM levels is enough to indicate that some form of microbial or bacterial activity was taking place.

Effluent and Salt Percentage. The only shrimp silage treatments to have measurable effluent were at the 37% DM level. The 10% molasses silage lost .002% of its volume as effluent, while the 20% molasses treatment lost .064% of it's volume as effluent. The effluents had a DM of 16.4% $\pm$ 1.41, a CP of 17.9% $\pm$ .47 on a DM basis, and a pH of 6.7 $\pm$ .06.

TABLE 3.1 Main effects of DM, molasses, and inoculant on the analyses of shrimp silage on day 40 post-ensiling (Experiment 1).

	DM, %				Molasses, %					Inoculant, %			
	37	47	52		0	10	20			0	.01		
Item				P-val				P-val	SEM+			SEM	P-val
DM, %	34.2[a]	43.5[b]	50.6[c]	<.0001	42.5	42.8	43.0	.4587	.3	43.0	42.6	.2	.2402
CP, %[1]	18.8[a]	15.3[b]	11.5[c]	<.0001	14.0[a]	15.8[b]	15.8[b]	<.0001	.1	15.2	15.2	.1	.6039
ADF, %[1]	21.3[a]	29.3[b]	34.9[c]	<.0001	32.2[a]	28.0[b]	25.2[c]	<.0001	.2	28.6	28.3	.2	.1034
ADIN[1,2]	.57[a]	.39[b]	.25[c]	<.0001	.38[a]	.41[b]	.41[b]	.0003	.01	.41	.39	.00	.0134
%ADIN[1,2]	19.0[a]	15.8[b]	13.7[c]	<.0001	16.8	15.8	16.0	.0160	.2	16.6	15.8	.2	.0033
EE, %[1]	1.8[a]	1.3[b]	1.4[b]	<.0001	1.5[a,b]	1.6[b]	1.5[a]	.0064	.0	1.5	1.5	.0	.2769
NH_3[3,4]	7.2[a]	6.6[b]	6.1[c]	<.0001	7.2[a]	6.4[b]	6.3[b]	<.0001	.1	6.7	6.6	.1	.3869
DMA[4]	185	297	308	<.0001	196	292	302	<.0001	17	274	252	14	.2617
TMA[4]	318	331	439	.0064	406	352	330	.1528	28	367	358	22	.7752
pH	6.8[a]	7.2[b]	6.9[a]	<.0001	7.4[a]	6.8[b]	6.7[b]	<.0001	.0	7.1	6.9	.0	<.0001
ACET, %[1]	2.46[a]	1.75[b]	1.24[c]	<.0001	2.17[a]	1.81[b]	1.47[c]	<.0001	.08	1.82	1.82	.07	.9841
PROP, %[1]	.34[a]	.20[b]	.09[c]	<.0001	.39[a]	.11[b]	.12[b]	<.0001	.01	.20	.21	.01	.5408
ISOB, %[1]	.08	.02	.02	.1267	.09	.02	.01	.0370	.02	.03	.05	.02	.5498
BUT, %[1]	.36[a]	.16[b]	.03[c]	<.0001	.38[a]	.11[b]	.07[b]	<.0001	.01	.17	.20	.01	.0033
ISOV, %[1]	.03[a]	.01[b]	.03[a]	<.0001	.06[a]	.00[b]	.00[b]	<.0001	.00	.03	.02	.00	<.0001
LACT, %[1]	7.67[a]	5.34[b]	1.58[c]	<.0001	2.23[a]	5.62[b]	6.73[c]	<.0001	.21	4.98	4.74	.17	.3334
VAL, %[1]	.02	.02	.01	.0266	.01	.02	.02	.1602	.00	.02	.02	.00	.4456
Odor[5]	1.9	1.8	1.8	.0624	3.0[a]	1.5[b,c]	1.1[c]	<.0001	.0	1.9	1.8	.0	<.0001

[a,b,c] Means within treatment in same row with different superscripts differ ($P < .01$).

+ SEM for both DM and molasses.

[1] DM basis. [2] ADIN = % N in ADF fraction, %ADIN = Percent of total N bound in the ADF fraction.

[3] Values for NH_3 are natural logarithms of original data. [4] Measurements in ppm. [5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 3.2 Interaction of molasses and inoculant on chemical analyses of shrimp silage at 37% DM on day 40 (Experiment 1).

	Level of molasses, %							
	0		10		20			
	Level of inoculant, %							
	0	.01	0	.01	0	.01		
Item							SEM	P-val
DM, %	33.2	32.3	34.5	34.5	35.4	35.3	.6	.6849
CP, %[1]	15.9	16.0	19.6	19.8	20.4	21.2	.3	.5178
ADF, %[1]	26.7	25.6	20.8	21.1	17.2	16.1	.4	.1635
ADIN[1,2]	$.57^{b,c}$	$.48^{a}$	$.56^{b}$	$.65^{c}$	$.60^{b,c}$	$.55^{a,b}$	.02	.0012
%ADIN[1,2]	22.5^{b}	$18.6^{a,c}$	$18.0^{a,c}$	$20.4^{b,c}$	$18.4^{a,c}$	16.2^{a}	.7	.0020
EE, %[1]	1.9	1.8	2.3	1.8	1.6	1.6	.1	.0207
NH_3[3,4]	8.5	7.8	6.7	6.7	6.3	6.9	.3	.0556
DMA[4]	110	148	246	238	163	205	21	.4342
TMA[4]	589	523	203	196	167	226	49	.4627
pH	7.7	7.4	6.8	6.7	6.3	6.2	.1	.5614
ACET, %[1]	3.72	3.57	1.70	1.63	2.06	2.08	.27	.9545
PROP, %[1]	.55	.67	.17	.16	.27	.24	.03	.0824
ISOB, %[1]	.12	.30	.02	.02	.02	.01	.10	.6229
BUT, %[1]	.71	.78	.09	.23	.09	.23	.04	.6631
ISOV, %[1]	$.10^{b}$	$.06^{a}$	$.00^{c}$	$.00^{c}$	$.00^{c}$	$.00^{c}$	.00	<.0001
LACT, %[1]	2.52	2.77	10.04	7.52	12.40	10.77	.67	.1486
VAL, %[1]	.05	.01	.01	.02	.00	.01	.01	.0207
Odor[5]	3.0^{a}	3.0^{a}	1.0^{c}	2.0^{b}	1.0^{c}	1.5^{d}	.1	<.0001

[a,b,c,d] Means in same row with different superscripts differ (P < .01).
[1] DM basis.
[2] ADIN = % N in ADF fraction, %ADIN = percent of total N bound in the ADF fraction.
[3] Values for NH_3 are natural logarithms of original data.
[4] Measurements in ppm.
[5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 3.3 Interaction of molasses and inoculant on chemical analyses of shrimp silage at 47% DM on day 40 (Experiment 1).

	Level of molasses, %							
	0		10		20			
	Level of inoculant, %							
	0	.01	0	.01	0	.01		
Item							SEM	P-val
DM, %	42.6	43.2	43.8	43.3	44.8	43.3	.4	.0531
CP, %[1]	14.1[a]	14.5[a]	15.9[b]	16.2[b]	16.1[b]	14.8[a]	.2	.0011
ADF, %[1]	32.9	32.4	28.4	28.7	27.0	26.4	.4	.4658
ADIN[1,2]	.38[a,b]	.33[a]	.37[a,b]	.43[b,c]	.45[c]	.37[a,b]	.01	.0014
%ADIN[1,2]	16.9[a,b]	14.2[a]	14.6[a]	16.4[a,b]	17.4[b]	15.7[a,b]	.6	.0068
EE, %[1]	1.4	1.5	1.2	1.2	1.3	1.4	.1	.6154
NH_3[3,4]	7.6	6.9	6.5	6.4	6.1	6.2	.2	.3398
DMA[4]	223	214	277	242	478	347	60	.5792
TMA[4]	280	311	217	372	396	412	94	.7254
pH	8.1[a]	7.4[b]	6.9[c]	6.9[c]	7.0[b,c]	6.8[c]	.1	.0044
ACET, %[1]	1.59	1.58	2.41	2.34	1.37	1.22	.18	.9335
PROP, %[1]	.42	.41	.15	.07	.05	.09	.02	.0659
ISOB, %[1]	.04[a]	.03[a,b]	.02[b,d]	.01[c]	.00[c]	.01[c,d]	.00	.0080
BUT, %[1]	.29[b]	.37[a]	.17[d]	.07[c]	.01[e]	.05[c,e]	.01	<.0001
ISOV, %[1]	.06[b]	.00[a]	.00[a]	.00[a]	.00[a]	.00[a]	.00	<.0001
LACT, %[1]	2.39	3.88	5.52	5.96	7.17	7.10	.50	.3232
VAL, %[1]	.00	.01	.03	.02	.04	.04	.01	.8460
Odor[5]	3.0	3.0	1.5	1.5	1.0	1.0	.1	.4625

[a,b,c,d] Means in same row with different superscripts differ ($P \leq .01$).

[1] DM basis.

[2] ADIN = % N in ADF fraction, %ADIN = percent of total N bound in the ADF fraction.

[3] Values for NH_3 are natural logarithms of original data.

[4] Measurements in ppm.

[5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 3.4 Interaction of molasses and inoculant on chemical analyses of shrimp silage at 52% DM on day 40 (Experiment 1).

	Level of molasses, %							
	0		10		20			
	Level of inoculant, %							
	0	.01	0	.01	0	.01		
Item							SEM	P-val
DM, %	50.5[a]	53.2[a]	52.5[a]	48.5[b]	49.3[a,b]	49.7[a,b]	.9	.0093
CP, %[1]	10.9[b]	12.4[a]	12.8[a]	10.4[b]	10.7[b]	11.7[a,b]	.3	<.0001
ADF, %[1]	38.0	37.7	34.2	34.7	32.6	32.1	.5	.6074
ADIN[1,2]	.26[a,c]	.26[a,c]	.26[a,c]	.21[b]	.24[c]	.27[a]	.01	<.0001
%ADIN[1,2]	15.2	13.2	12.5	12.9	14.2	14.4	.4	.0125
EE, %[1]	1.2	1.2	1.6	1.4	1.4	1.5	.1	.1210
NH_3[3,4]	6.4	6.1	6.0	6.2	6.0	6.1	.1	.1116
DMA[4]	232[a]	249.[a]	482[b]	264[a]	258[a]	364[a,b]	31	.0007
TMA[4]	318[a]	411[a]	773[b]	350[a]	361[a]	421[a]	49	.0003
pH	7.1	6.9	6.9	6.7	7.0	7.1	.1	.0827
ACET, %[1]	.83[b]	1.75[a]	1.73[a]	1.08[a,b]	.99[b]	1.08[a,b]	.16	.0012
PROP, %[1]	.08[b,c]	.20[a]	.12[c]	.03[b]	.05[b]	.05[b]	.01	<.0001
ISOB, %[1]	.03[a]	.04[a]	.03[a]	.00[b]	.00[b]	.01[b]	.00	<.0001
BUT, %[1]	.05	.06	.04	.02	.01	.00	.01	.3219
ISOV, %[1]	.09	.07	.00	.00	.00	.02	.01	.1508
LACT, %[1]	.74	1.09	2.30	2.40	1.73	1.20	.28	.2981
VAL, %[1]	.00	.00	.01	.01	.02	.01	.01	.4850
Odor[5]	3.0	3.0	1.5	1.5	1.0	1.0	.1	<.6845

[a,b,c,d] Means in same row with different superscripts differ (P < .01).
[1] DM basis.
[2] ADIN = % N in ADF fraction, %ADIN = percent of total N bound in the ADF fraction.
[3] Values for NH_3 are natural logarithms of original data.
[4] Measurements in ppm.
[5] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

The percentage of NaCl in the shrimp silage decreases as the level of DM increased. The 37, 47, and 52% DM silages had 5.86 $\pm$ 1.21, 3.99 $\pm$ 1.41, and 3.09 $\pm$ .96 NaCl, % respectively. The decreases can be attributed to a dilution of the salt-preserved crab waste as straw is added.

The results indicate that a successful lactic acid fermentation took place with the salt-preserved shrimp waste. The high quality silages were produced at the 47% DM, with the addition of either 10 or 20% molasses, and at 52% DM at 10% molasses. Shrimp waste combined with grass straw and molasses at those conditions produced a silage with VFA and lactic acid levels similar to a normal corn or grass silage. The highest quality silage was the 47% DM, 20% molasses non-inoculated treatment. With the exception of a high pH, this silage exhibited the characteristics of a normal lactic acid fermentation and was the highest in CP.

Experiment 2. Ensiling Salt-Preserved Crab Waste with Grass Straw, Molasses and Inoculant

DM and CP changes. Analysis of main effects of DM, molasses and inoculant on crab silage on day 40 yielded a high F-value and a significant ($p < .0001$) molasses effect for most variables. Therefore, the data was analyzed separately at each of the three molasses levels.

Crab silages with initial DM of 43.46 and 52% had a decrease in DM on day 40 of 1.8, 1.5 and 2.4 percentage points respectively (Table 3.5). As the level of molasses increased from 0 to 10%, DM significantly decreased, but as molasses increased from 10 to 20%, DM significantly increased. The addition of inoculant significantly decreased DM% (Table 3.5). There was a significant DM by inoculant interaction at 0% molasses (Table 3.6). Dry matter percentage remained unchanged with the addition of inoculant at the 43% DM, but DM% decreased at both the 46 and 52% DM with the addition of inoculant (Table 3.6). The changes in DM%, although statistically significant, are numerically small and most of the differences

were present in the starting point crab silage. This indicates that little change in DM% took place during the 40 day ensiling experiment.

CP% significantly increased as the DM increased and significantly decreased as the level of molasses increased (Table 3.5). The increase in CP% can be accounted for by the fact that water was added to all of the 43% DM silages and some of the 46% DM silages. So a decrease in added water would lead to an increase in CP%.

ADF% decreased significantly by 1.9 percentage points as DM increased from 43 to 52%. Acid detergent fiber% decreased by 4.9 percentage points as molasses increased from 0 to 20% (Table 3.5).

ADIN and %ADIN, which are indicators of the level of chitin, decreased significantly as the DM level increased, as the molasses level increased and as inoculant was added (Table 3.5). The largest portion of the ADIN and %ADIN is the chitin in the crab waste, so as grass straw, molasses and inoculant are added, it can be expected that the both ADIN and %ADIN would decrease.

Ether extract % increased significantly as the level of DM increased and as the level of molasses increased (Table 3.5).

The ammonia concentration decreased significantly as the level of DM increased and with the addition of molasses (Table 3.5). There was a significant DM by inoculant interaction at 20% molasses. The addition of inoculant decreased the ammonia concentration at 52% DM but failed to have an effect at 43 and 46% DM (Table 3.8). It is possible that the 20% molasses level was the minimum amount needed to elicit a response to the addition of inoculant.

The DMA and TMA concentrations both increased as the level of DM increased and the DMA concentration increased as the level of molasses increased (Table 3.5). There was a significant DM by inoculant interaction for DMA and TMA at both the 10 and 20% molasses levels. At 10% molasses, the addition of inoculant at 43% DM decreased the DMA concentration, had no effect at the 46% DM and increased DMA concentration 52% DM (Table 3.7). Also at 10% molasses, the TMA concentration was not affected by the addition of inoculant at 43 and 46% DM, but was increased at 52% DM (Table 3.7). At 20% molasses, the DMA and TMA

concentrations both were not affected by the addition of inoculant at 43 and 46% DM, but both decreased at 52% DM (Table 3.8). Addition of inoculant should enhance fermentation and promote preservation of the silage which should lower the DMA and TMA concentrations. It appears that 20% molasses was needed as a minimum to receive an inoculant response.

Ensiling Variables. The pH decreased significantly with the addition of molasses. There was a significant DM by inoculant interaction at 20% molasses for pH. The pH of crab silage was not affected by the addition of inoculant at 43% DM, but was decreased at 46 and 52% DM (Table 3.8). Again indicating that 20% molasses was needed to elicit an inoculant response.

Acetic acid concentration decreased by .5 percentage points as the level of molasses increased from 0 to 20%. Lactic acid concentration decreased by 1.1 percentage points as the level of DM increased from 43 to 52% and increased with the addition of molasses. All other VFA's tended to decrease or stay the same as the level of DM increased, as the molasses level increased, and with the addition of inoculant.

Odor evaluation scores were improved with the addition of molasses. The silages in this experiment to receive good odor evaluation scores were the 10% molasses, 52% DM inoculated and non-inoculated silage and the 20% molasses, 46% DM non-inoculated silage.

The average room temperature, maximum, and minimum temperatures were 5.7, 11.0, and 2.4°C, respectively. The mini-silo temperature for the 0, 10, and 20% molasses silages were 5.6, 5.6, and 5.8°C, respectively. It is known that the effect of ambient temperature on the ensiling temperature is related to the mini-silo size (Parker, 1978). The lack of variance in mini-silo temperatures, from room temperature, at all three molasses levels is inconclusive as to whether or not some form of microbial or bacterial activity was taking place.

Effluent and Salt Percentage. The crab silage treatments with measurable effluent were at the 10 and 20% molasses levels. At 10% molasses, the 43% DM treatment

lost .005% of its volume as effluent. At 20% molasses, the 43% DM silage lost .03% of it's volume as effluent while at 46% DM only .004% was lost. The effluents had a DM of 17.1% $\pm$ 1.10, a CP of 16.3% $\pm$ .54 and a pH of 6.6 $\pm$ .12. Selected effluent samples which were analyzed for BOD were all greater than 10,000 mg/L O_2.

TABLE 3.5 Main effects of DM, molasses, and inoculant on the analyses of crab silage on day 40 post-ensiling (Experiment 2).

	DM, %				Molasses, %					Inoculant, %			
	45	50	55		0	10	20			0	.01		
Item				P-val				SEM+	P-val			SEM	P-val
DM, %	41.2[a]	44.4[b]	49.6[c]	<.0001	45.1[a]	44.3[b]	45.8[c]	.2	<.0001	45.6	44.5	.1	<.0001
CP, %[1]	18.7[a]	20.2[b]	19.9[b]	<.0001	21.3[a]	20.2[b]	17.3[c]	.2	<.0001	19.5	19.7	.1	.5501
ADF, %[1]	22.2[a]	20.1[b]	20.3[b]	<.0001	23.6[a]	20.2[b]	18.7[c]	.2	<.0001	21.0	20.7	.2	.2178
ADIN[1,2]	.47[a]	.46[a]	.41[b]	.0006	.55[a]	.46[b]	.34[c]	.01	<.0001	.47	.43	.01	.0013
%ADIN[1,2]	15.6[a]	14.8[a,b]	13.5[b]	.0028	16.4[a]	14.7[b]	12.8[c]	.4	<.0001	15.3	14.0	.3	.0059
EE, %[1]	1.6[a]	2.0[b]	2.0[b]	<.0001	1.7[a]	1.9[b]	1.9[b]	.1	.0060	1.8	1.9	.0	.0621
NH_3[3]	600[a]	402[b]	357[b]	<.0001	775[a]	270[b]	314[b]	20	<.0001	468	438	16	.1922
DMA[3]	168[a]	240[a]	579[b]	<.0001	251[a]	284[a]	452[b]	20	<.0001	331	327	16	.8485
TMA[3]	362[a]	388[a]	806[b]	<.0001	514	432	610	50	.0567	577	460	41	.0519
pH	7.0	6.9	6.9	.2000	7.5[a]	6.7[b]	6.7[b]	.0	<.0001	7.0	7.0	.0	.6163
ACET, %[1]	1.14	1.43	1.52	.0574	1.50[a]	1.57[a]	1.01[b]	.11	.0019	1.46	1.26	.09	.1296
PROP, %[1]	.03	.02	.02	.5516	.03	.03	.02	.00	.1951	.02	.02	.00	.7696
ISOB, %[1]	.01	.00	.01	.6094	.01	.01	.00	.00	.0160	.01	.01	.00	.9246
BUT, %[1]	.01	.00	.00	.2171	.00[a]	.00[a]	.01[b]	.00	.0014	.00	.01	.00	.2880
ISOV, %[1]	.01	.01	.02	.5100	.03[a]	.01[b]	.00[b]	.00	<.0001	.01	.01	.00	.4257
LACT, %[1]	2.26[a]	2.05[a]	1.05[b]	<.0001	.29[a]	2.73[b]	2.33[b]	.13	<.0001	1.79	1.78	.11	.9724
VAL, %[1]	.00	.01	.01	.0139	.00[a]	.01[b]	.00[a]	.00	<.0001	.01	.00	.00	.1947
Odor[4]	2.0[a]	2.1[a]	2.0[a]	.0026	2.8[a]	1.4[b]	1.8[c]	.0	<.0001	2.0	2.0	.0	.0319

[a,b,c] Means within treatment in same row with different superscripts differ (P < .01).
+ SEM for both DM and molasses.
[1] DM basis. [2] ADIN = % N in ADF fraction, %ADIN = Percent of total N bound in the ADF fraction.
[3] Measurements in ppm. [4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 3.6 Least square means of crab silage characteristics for the interaction of DM and inoculant at 0% molasses on day 40 (Experiment 2).

	Level of DM, %							
	43		46		52			
	Level of Inoculant, %							
	0	.01	0	.01	0	.01		
Item							SEM	P-val
DM, %	40.4[a]	39.7[a]	46.2[b]	43.8[c]	52.0[d]	48.6[e]	.4	.0092
CP, %[1]	21.6	21.8	22.3	22.8	19.1	20.2	.4	.4614
ADF, %[1]	23.4	24.9	21.0	20.6	26.3	25.2	.6	.1249
ADIN[1,2]	.72	.60	.55	.53	.45	.44	.02	.1198
%ADIN[1,2]	21.2	17.6	15.7	14.8	15.2	13.9	.9	.3244
EE, %[1]	1.5	1.6	2.0	1.9	1.6	1.7	.1	.4891
NH_3[3]	1081	926	526	893	538	682	79	.0196
DMA[3]	42	76	214	295	456	423	39	.3794
TMA[3]	396	422	520	523	632	593	135	.9712
pH	7.2	7.9	7.5	7.7	7.0	7.2	.8	.1646
ACET, %[1]	1.43	1.32	1.37	1.30	1.54	2.02	.17	.2174
PROP, %[1]	.01	.02	.05	.02	.03	.01	.01	.1647
ISOB, %[1]	.01	.02	.01	.01	.01	.02	.01	.9792
BUT, %[1]	.00	.00	.00	.01	.00	.00	.00	.8345
ISOV, %[1]	.02	.03	.02	.03	.02	.03	.01	.9274
LACT, %[1]	.22	.29	.25	.19	.31	.48	.18	.8181
VAL, %[1]	.00	.00	.00	.00	.00	.00	.00	.3966
Odor[4]	2.5	2.5	3.0	3.0	3.0	3.0	.1	.6872

[a,b,c,d,e] Means in same row with different superscripts differ ($P < .01$).
[1] DM basis.
[2] ADIN = % N in ADF fraction, %ADIN = percent of total N bound in the ADF fraction.
[3] Measurements in ppm.
[4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 3.7 Least square means of crab silage characteristics for the interaction of DM and inoculant at 10% molasses on day 40 (Experiment 2).

	Level of DM, %							
	43		46		52			
	Level of Inoculant, %							
	0	.01	0	.01	0	.01		
Item							SEM	P-val
DM, %	41.5	40.9	43.5	44.0	47.5	43.8	.2	.0193
CP, %[1]	19.4	18.6	21.2	21.1	20.6	20.4	.5	.7652
ADF, %[1]	22.6	22.2	19.8	19.6	19.4	17.9	.4	.2761
ADIN[1,2]	.54	.40	.47	.48	.44	.42	.03	.0466
%ADIN[1,2]	17.9	13.8	14.3	14.7	13.8	13.6	1.1	.1263
EE, %[1]	1.7	1.7	2.0	2.1	1.9	2.3	.1	.4426
NH_3[3]	403	381	215	237	169	213	24	.3997
DMA[3]	207[a]	119[b]	240[a]	197[a]	341[c]	602[c]	14	<.0001
TMA[3]	498[a,b]	240[a]	500[a,b]	339[a]	293[a]	723[b]	87	.0040
pH	6.7	6.5	6.6	6.6	6.8	6.9	.1	.0741
ACET, %[1]	1.15	1.10	2.48	1.68	1.75	1.28	.39	.6451
PROP, %[1]	.03	.04	.01	.02	.02	.04	.01	.7949
ISOB, %[1]	.00	.00	.01	.00	.02	.00	.01	.5052
BUT, %[1]	.00	.01	.00	.00	.00	.00	.00	.7880
ISOV, %[1]	.01	.00	.02	.01	.01	.01	.00	.6790
LACT, %[1]	3.84	3.33	2.95	2.86	2.10	1.28	.45	.7150
VAL, %[1]	.01	.01	.01	.01	.02	.01	.00	.3067
Odor[4]	1.2	1.2	2.0	2.0	1.0	1.0	0	.6186

[a,b,c] Means in same row with different superscripts differ ($P \leq .01$).
[1] DM basis.
[2] ADIN = % N in ADF fraction, %ADIN = percent of total N bound in the ADF fraction.
[3] Measurements in ppm.
[4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

TABLE 3.8 Least square means of crab silage characteristics for the interaction of DM and inoculant at 20% molasses on day 40 (Experiment 2).

	Level of DM, %							
	43		46		52			
	Level of Inoculant, %							
	0	.01	0	.01	0	.01		
Item							SEM	P-val
DM, %	43.0	41.9	44.6	44.2	51.6	49.6	.5	.2676
CP, %[1]	15.9	15.1	16.8	17.0	19.0	20.0	.4	.0812
ADF, %[1]	19.6	20.3	20.4	19.0	16.4	16.6	.3	.0154
ADIN[1,2]	.29	.26	.39	.36	.36	.37	.02	.5106
%ADIN[1,2]	12.1	11.2	15.3	13.8	12.5	12.2	1.0	.8257
EE, %[1]	1.6	1.7	1.7	2.1	2.2	2.2	.1	.3944
NH_3[3,4]	426[a]	380[a,b]	254[c,d]	288[c]	330[b,c]	209[d]	134	.0004
DMA[4]	200[a]	365[a,c]	208[a]	286[a]	1074[b]	579[c]	73	.0013
TMA[4]	266[a]	351[a]	208[a]	240[a]	1882[b]	712[a]	140	.0011
pH	6.5[a]	6.6[a]	6.6[a]	6.7[b]	7.2[c]	6.9[b]	.1	.0044
ACET, %[1]	1.10	.71	1.17	.56	1.14	1.35	.21	.1782
PROP, %[1]	.03	.02	.03	.02	.01	.01	.00	.3882
ISOB, %[1]	.00	.00	.00	.00	.00	.00	.00	.5853
BUT, %[1]	.01	.02	.01	.01	.01	.01	.01	.7869
ISOV, %[1]	.00	.00	.00	.00	.01	.01	.00	.5781
LACT, %[1]	2.50	3.34	3.24	2.79	.65	1.46	.29	.0758
VAL, %[1]	.00	.00	.01	.00	.00	.01	.00	.0476
Odor[4]	2.2	2.2	1.0	1.5	2.0	2.0	.1	.0348

[a,b,c,d] Means in same row with different superscripts differ ($P \leq .01$).

[1] DM basis.

[2] ADIN = % N in ADF fraction, %ADIN = percent of total N bound in the ADF fraction.

[3] Measurements in ppm.

[4] Odor evaluation, 1 = good, 2 = questionable, 3 = poor.

The %NaCl in the crab silages decreased as the level of molasses increased. The %NaCl at 0, 10, and 20% molasses were 5.70% $\pm$ 2.06, 5.32% $\pm$ 1.32, and 4.09% $\pm$ 1.16, respectively. The addition of molasses served as a dilutant and lowered the %NaCl in the silages.

These results indicates that lactic acid fermentation took place with the salt-preserved crab waste. The higher quality silages produced in this experiment were at 46% DM with 10 or 20% molasses, and 52% DM at 10% molasses. Although the pH and acetic and lactic acid concentrations did vary from those of a normal silage, the waste appeared to have undergone an adequate fermentation. Abazinge et al. (1994) found intakes of 58% DM crab waste silages treated with acetic acid or molasses to be similar to the basal diet when fed to sheep. The best crab silage produced in this experiment was the 46% DM, 10% molasses inoculated silage.

Experiment 3. Ruminal Degradability of Shrimp and Crab Waste or Shrimp and Crab Silage

The onset of ruminal degradation of nutrients as measured by lag time, were shorter for shrimp waste than crab waste. Shrimp waste contained a smaller undegradable portion of DM, CP and ADIN than crab waste, but a higher undegradable fraction of ADF. The rate of degradation was faster for shrimp waste in all nutrients except CP, in which crab and shrimp waste had the same rate of degradation. The amount of nutrient available in the rumen is lower in shrimp waste for DM and CP but higher than crab waste in ADF and ADIN.

Dry matter, CP, and ADF of crab silage had a shorter lag time than the same nutrients in shrimp silage; the ADIN lag time in shrimp silage was longer than in crab silage. There was a higher portion of undegradable DM, CP, and ADF in shrimp silage than crab silage, however the undegradable ADIN fraction was lower for shrimp silage. The rate of degradation of DM and CP was faster in shrimp silage, while the rate of degradation for ADF and ADIN was the same for both wastes. The ruminally available nutrient was similar between silages however, shrimp silage had a slightly higher availability for all four fractions measured.

The ensiled shrimp and crab waste had a longer lag time for DM, a shorter lag time for ADF and ADIN. The lag time for CP for crab silage was shorter than either waste. While the lag time for shrimp silage was shorter than the fresh crab waste, it was longer than the lag time for fresh shrimp waste.

Ensiled shrimp had a higher portion of undegradable DM, CP, ADF and ADIN than the fresh shrimp waste. Ensiled crab had a lower portion of undegradable DM than fresh crab waste but had a higher undegradable portion of CP, ADF, and ADIN than fresh crab waste.

Ensiled shrimp waste had a faster rate of degradation for DM and CP, slower for ADF, but no different from fresh shrimp waste in rate of degradation of ADIN. Ensiled crab waste had the same rate of degradation of DM as fresh crab waste, but had a faster rate of degradation for CP, ADF and ADIN.

The shrimp and crab silages both had higher percentages of ruminally available DM and CP, but lower percentages of ruminally available ADF and ADIN than fresh shrimp or crab waste.

A small decrease in ruminally available ADF and ADIN can be expected in the ensiled waste as a result of diluting the fresh waste with grass straw and molasses. The increase in ruminally available CP can be attributed to either a grass straw with highly available CP or a breakdown of the chitin in the ensiling process. The process of ensiling could enhance the degradation of the chitin molecule and release the nitrogen from it's structure. This would increase the ruminally available CP and decrease the ruminally available ADIN. The data suggests that the process of ensiling can increase the ruminally available CP and decrease the ruminally available ADIN. This suggests that proper ensiling promotes degradation of the chitin molecule and its availability to the ruminal microflora.

TABLE 3.9 Nutrient ruminal degradability of fresh shrimp or crab waste and shrimp or crab silage with two steers over 9 time points from 0 to 96 hr (Experiment 3).[1]

	Waste		Silage	
	Shrimp	Crab	Shrimp[3]	Crab[4]
Lag time, hr.				
DM	-.5	-.3	3.9	.8
CP	1.1	2.0	1.5	.4
ADF	6.9	8.3	5.3	2.1
ADIN	5.3	6.0	2.3	3.0
Undegradable, %				
DM	25.0	31.5	32.9	28.4
CP	10.0	12.4	18.7	17.7
ADF	20.0	16.4	45.9	41.2
ADIN	4.1	11.9	38.0	45.1
Rate of degradation, k, fraction/hr.				
DM	.04	.03	.05	.03
CP	.03	.03	.05	.04
ADF	.04	.02	.03	.03
ADIN	.03	.02	.03	.03
Ruminal available nutrient, %[2]				
DM	36.9	38.9	54.7	53.3
CP	43.0	53.3	68.4	64.1
ADF	44.2	41.0	28.5	27.6
ADIN	43.6	33.0	31.3	29.1

[1] ADF and ADIN for crab waste are results from only one steer.
[2] Assume a rate of liquid passage of .04/hr.
[3] 47% DM, 20% molasses, non-inoculated shrimp silage.
[4] 46% DM, 10% molasses, inoculated crab silage.

CONCLUSION

Previous work has shown that fresh shrimp and crab waste can be quickly and economically preserved with 7.5% salt. The objective of this research was to utilize other by-products and transform the salt-preserved shrimp and crab waste into a seafood silage. This would increase its manageability and palatability as a feedstuff for dairy heifers in Oregon's coastal areas. Successful results were achieved from ensiling the salt-preserved shrimp and crab waste with grass straw, molasses and inoculant in the 46 to 52% DM range. The ensiled products were high in CP and had a good silage odor.

The ruminal degradability trial indicated that shrimp and crab waste are highly digestible in the rumen, and that the process of ensiling can increase the digestibility of the preserved wastes.

The process of ensiling is low cost and utilizes other by-products to produce a high quality silage which is economical for incorporation into dairy heifer rations. Further work needs to be done to determine at what rate either of the seafood silages could be incorporated in the ration and to further test the digestibility of this product. Shrimp and crab waste continue to have potential as a dairy heifer feedstuff.

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SUMMARY

Protein degradation and ammonia production occur rapidly in seafood waste products making speed of preservation vital to maintaining the level of protein in the fresh wastes. During Experiments 1, 2, and 3 (Chapter 2), a lactic acid fermentation of the wastes was attempted. There were indications that a fermentation began, but ended before reaching the desired pH range and a stable product. This can be attributed to either the rapid deterioration of the wastes, a lack of fermentable carbohydrates, the high buffering capacity of the wastes, or a combination of the three. Molasses at greater than 25% may decrease the pH in the waste, however this would become cost prohibitory and the fermentation process would still take at least 6 days. In an attempt for a quicker, lower cost preservation, Experiments 4 and 5 (Chapter 2) were conducted to test salt as a preservative. Salt, at a 7.5% level or greater, in both wastes, produced a product with good odor, minimal ammonia production, and a high level of preserved crude protein. This was a low cost method to preserve and store crustacean waste which minimized protein degradation and odor production.

The objectives of Experiments 1 and 2 (Chapter 3) were to utilize other by-products and transform the salt-preserved shrimp and crab waste into a seafood silage. This would increase its manageability and palatability as a feedstuff for dairy heifers in Oregon's coastal areas. Successful results were received from ensiling the salt-preserved shrimp and crab waste with grass straw, molasses and inoculant in the 46 to 52% DM range. The ensiled products were high in CP and had a good silage odor. When offered to four cows in a completely randomized 4 X 4 Latin square palatability trial, the cows consumed most of the shrimp silage offered at 47 and 52% DM while rejecting the 37% DM shrimp silage.

The ruminal degradability trial indicated that fresh shrimp and crab waste were highly digestible in the rumen, and that the process of ensiling can increase the digestibility of the preserved wastes.

The combination of preserving and then ensiling crab and shrimp waste could help to eliminate the current disposal problems in coastal areas as well as provide a high protein feedstuff to dairy heifers in Oregon at a cost comparable to conventional feedstuffs. This form of recycling natural resources, and utilizing other industry by-products will continue to gain popularity as the environmental restrictions on those who manage natural resources continue to grow.

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APPENDICES

APPENDIX A

ANALYSIS OF SILAGE QUALITY ON THE GAS CHROMATOGRAPH MEASUREMENT OF THE VOLATILE FATTY ACIDS AND LACTIC ACID

Materials needed:

* 250 ml wide-neck bottles with caps
* Precision Instruments Shaker
* Whatman No. 1 filter paper
* Wide-top funnels
* 100 ml beakers
* 10 ml test tubes
* Centrifuge
* 1 ml pipette
* 4 ml pipette
* Oxalic acid, .12 M
* Gas Chromatograph injection vials
* 7 ml plastic storage vials
* VFA, and Lactic acid standards
* HP 5890 Gas Chromatograph

VOLATILE FATTY ACIDS AND LACTIC ACID ANALYSIS

When attempting to assess silage quality it is important to determine the levels of the volatile fatty acids and lactic acid in the silage. The following procedure is less time consuming than the classical distillation method and HPLC procedures and has excellent recovery. For best results the silage sample should be frozen immediately after sampling and then thawed within 10 hours of extraction. The prepartion procedure takes about three hours for 12 samples. This procedure has been modified from the following reference: Fussell, R. J., D. V. McCalley. Sept. 1987. Determination of Volatile Fatty Acids (C_2-C_5) and Lactic Acid in Silage by Gas Chromatography. Analyst. Vol 112. Pgs. 1213-1216.

Procedures for preparation:

1. Transfer 20 g of sample into a labelled 250 ml wide-necked bottle and add 100 mL of distilled water.

2. Cap tightly and fasten very securely into the shaker. Sandwiching paper towels between each row of bottles will prevent rattling and breakage.

3. Shake on high for one hour.

4. Assemble filter apparatus with funnels, beakers and filter paper.

5. Filter sample through Whatman No. 1 filter paper. Allow to filter until at least 50 mls have been collected.

6. Pipette 4 ml of filtrate into a labelled test tube.

7. Add 2 mls of .12 M oxalic acid and 2 mls of distilled water.

8. Cover top of test tube and invert three times to agitate.

9. Place test tubes into the centrifuge and centrifuge at 3500rpm (2600g) for 5 minutes.

10. Pipette 1 ml of supernatant into a labelled GC vial and cap it. If GC vial will not be injected within 48 hours then freeze until ready for use.

11. Pour remaining supernatant into a 7 ml plastic vial, cap tightly, label and freeze.

12. Remove labels from glassware and wash all glassware.

13. Refreeze all silage samples.

14. This procedure produces a 10 part dilution.

Procedure for .12 M Oxalic acid:

1. Add 1.08048 grams of Oxalic acid (MW - 90.04) to a 100 ml volumetric flask.

Procedure for preparation of standards:

1. VFA and Lactic acid levels are generally reported on a dry matter percent.

2. Lactic acid generally ranges from 2.1 - 8.5%.
   Acetic acid ranges from .5 - 2.0%.
   Butyric acid is generally less than 0.1%.
   Proprionic acid ranges from 0 - 1%.

3. Thaw a vial of prepared VFA standards and add the following to a 8 ml test tube:
   3.3 mls distilled H2O
   .1 ml VFA standards
   .2 mls Lactic acid (25%)
   .4 mls Oxalic acid (.3M)

4. Vortex for 1 minute and then pipette 1 ml into each of 4 GC vials, label, cap and freeze.

This provides the following acids at the following weight percents and retention times:

	Wt. Percent	Retention Times
Acetic :	.1576%	1.8
Proprionic :	.0496%	3.8
Isobutyric :	.0266%	6.7
Butyric :	.04944%	9.0
Isovaleric :	.02486%	17.6
Lactic :	.276%	18.8
Valeric :	.02399%	22.1

Formula to convert to % dry matter:

Weight percent of acid * dilution factor (10) / dry matter percent of sample:

For example assume: wt. % of acid to be .0234,
dilution factor is 10
dry matter is 33.5%

.0234 * 10 / .335 = .699% acid

The software package on the GC computer has an automatic multiplier to simplify this process. For each sample the multiplier will be different as each sample will have a different dry matter. Divide the dilution factor (10) by the dry matter % and use this factor as the multiplier. In the above example the multiplier would be 29.85.

Methods for analysis on the GC:

The current method is "Silage. MTH"
Flow rate: 24 mls / minute
Column temperature: 165
Injector temperature: 220
Detector temperature: 220

If the level of lactic acid in the silage is high enough to cause carry over it may be necessary to increase the dilution. To maintain column sensitivity it is helpful to run a distilled water rinse with .03 M Oxalic acid between each sample.

APPENDIX B

PREPARATION OF SEAFOOD SILAGE SAMPLES FOR ANALYSIS OF DMA AND TMA ON THE GAS CHROMATOGRAPH

Materials needed:

* Same materials as for measurement of VFA's and lactic acid

TRIMETHYLAMINE AND DIMETHYLAMINE ANALYSIS

The measurement of levels of DMA and TMA allows the ability to determine the degree of deterioration of the seafood waste fraction of seafood silages.

Procedures for preparation of samples:

1. Follow steps 1 - 5 in the preparation of silages for VFA and Lactic acid analysis. The filtrate from one silage sample can be used for both VFA and lactic acid analysis as well as DMA and TMA analysis.
2. Pipette 4 ml of filtrate into a test tube.
3. Add 4 mls of distilled water to the test tube.
4. Invert test tube three times to agitate.
5. Centrifuge at 3500 rpm (2600 x g) for 5 minutes.
6. Pipette 1 ml of supernatant into a labelled GC vial, cap and freeze.
7. Pour the remaining supernatant into a plastic storage vial, cap tightly, label and freeze.
8. Clean all used glassware.
9. Refreeze all silage samples.
10. This procedure produces a 10 part dilution.

Procedure for preparation of standards:

1. DMA and TMA are generally reported on a ppm basis and normally range from 50 to 1000 ppm on seafood silages. Values as high as 2000 can be found on straight seafood wastes.

2. A 25 ppm or 50 ppm standard should allow for accurate area integrations on seafood silages. On seafood wastes a 50 ppm or 100 ppm standard may be more accurate.

3. To make a 100 ppm standard of both DMA and TMA: Add 0.377 grams of DMA and 0.337 grams of TMA to 2000 mls.

4. The dilution factor of 10 can again be inserted as a multiplier as was done for the VFA and lactic acid analysis.

Methods for analysis on the GC:

The current method is "DMA&TMA.MTH"
Flow rate: 18 mls/minute
Column temperature: 70
Injector temperature: 180
Detector temperature: 180

Depending on the levels of contaminants in the sample it may be necessary to run a rinse between each sample and raise the column temperature up to around 150 degrees Celcius.

APPENDIX C

PREPARATION OF SEAFOOD SILAGE SAMPLES FOR ANALYSIS OF NaCl USING QUANTABS

Materials needed:

* Quantab Titrators, Chloride, Enviornmental test systems, Inc, Elkhardt, IN 46514.

* Same materials as for measurement of VFA's and lactic acid

* A frozen extract from either the VFA and lactic analysis or the DMA and TMA analysis can be used to measure NaCl.

NaCl ANALYSIS

The measurement of levels of NaCl in seafood silages allows the ability to balance minerals in diets containing seafood silages.

Procedures for preparation of samples:

1. Thaw the frozen silage extracts in the 7 ml plastic vials.

2. Once thawed insert the lower end of the Quantab stick into the sample in the vial.

3. Leave the strip in the solution until it becomes saturated and the signal string turns black.

4. The Quantab unit can be determined between 2 - 30 minutes after the signal string turns black.

5. The Quantab unit, read to the nearest tenth, can then be converted to percent NaCl using the calibration table included with the Quantabs.

6. The true percent NaCl can be obtained by multiplying the percent NaCl from the table by 10 to account for the dilution factor.

7. The Quantab strips can be disposed of and all vials should be re-capped and returned to the freezer.

APPENDIX D

ECONOMIC ANALYSES OF SEAFOOD SILAGES

Costs of ensiling 100 ton of shrimp or crab silage:

Shrimp silage:

54.3 tons shrimp waste ($7.00/ton)	=	380.10
24.6 tons grass straw ($25.00/ton)	=	615.00
16.7 tons molasses @ $120.00/ton	=	2004.00
4.4 tons salt @ $60.00/ton	=	264.00

Shrimp silage cost of inputs per ton = $ 32.63

Crab silage:

64.9 tons shrimp waste ($7.00/ton)	=	454.00
13.2 tons grass straw ($25.00/ton)	=	330.00
10.0 tons molasses @ $120.00/ton	=	1200.00
5.3 tons salt @ $60.00/ton	=	318.00

Crab silage cost of inputs per ton = $ 23.02

Relative Feed Value:

On CP basis, both silages are similar to alfalfa silage for crude protein values, therefore .080 was used as the soybean constant. Using an average of the five Cornell TDN equations for forage (based on ADF), the values were similar to corn silage for TDN. Therefore .265 was used as the corn constant. The June, 1994 price of soybean meal was $242/ton and corn was $129/ton. A Relative Feed Value of $53.55/ton. was calculated for both silages.

Cost per kg of crude protein:

Calculation of cost per kg of CP on a DM basis;

Crab silage	$0.15/kg of CP
Shrimp silage	$0.30/kg of CP
Clover silage	$0.76/kg of CP
Grass silage	$0.80/kg of CP

Crab and shrimp silage are lower cost on a CP basis than grass or clover silage.